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Molecular Cloning and Expression in *Escherichia coli* K-12 of Chromosomal Genes Determining the O7 Lipopolysaccharide Antigen of a Human Invasive Strain of *E. coli* O7:K1

MIGUEL A. VALVANO†* AND JORGE H. CROSA

Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon 97201

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We have cloned and studied the expression in *Escherichia coli* K-12 of chromosomal *rfb* genes determining the biosynthesis of the O7 lipopolysaccharide (LPS) antigen from *E. coli* K1 strain VW187. Two *E. coli* K-12 strains carrying recombinant cosmids gave positive coagglutination reactions with protein A-rich staphylococcal particles bearing an O7-specific rabbit polyclonal antiserum. Silver-stained polyacrylamide gels of total membranes extracted with hot phenol showed O side chain material which had O7 specificity as determined by immunoblotting experiments. However, the amount of O7 LPS expressed in *E. coli* K-12 was considerably lower than that produced by the wild-type strain VW187. Deletion and transposition experiments identified a region of about 17 kilobase pairs which is essential for the expression of O7 LPS. The existence of homologies between the O7 LPS genes and other *E. coli* O side chain genes was investigated by Southern blot hybridization experiments. An O7-specific probe fragment of 15 kilobase pairs did not hybridize to genomic DNA digests of *E. coli* strains belonging to several different O types, demonstrating that the O7 LPS genes are unique.

Lipopolysaccharide (LPS) is a complex surface molecule located in the outer membrane of gram-negative bacteria. It consists of three moieties: lipid A, core oligosaccharide, and O-specific antigen or O side chain (18, 20, 23). The O antigen, consisting of a linear polymer of oligosaccharide repeating units, is the most external LPS component. The possession of O antigen endows pathogenic bacteria with the property to resist phagocytosis and lysis by complement (4, 7, 25, 26).

Escherichia coli O7:K1 strains are the most common etiological agents of neonatal septicemia and meningitis (1, 12, 15, 26) as well as of urinary tract infections in older patients (15). These virulent strains are serum resistant due to the possession of both a K1 capsule and O7 LPS, which mediate resistance to lysis by the alternative and classical pathways of serum complement, respectively (25).

In this article, we report the cloning and expression in *E. coli* K-12 of O7 LPS genes from the septicemic *E. coli* O7:K1 isolate VW187, and we also present evidence that these genes do not show homologies with genomic DNA of *E. coli* strains belonging to several different O types.

MATERIALS AND METHODS

Materials, bacterial strains, and plasmids. Chemicals and antibiotics used in this study were from Sigma Chemical Co., St. Louis, Mo. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md., and were used according to the conditions suggested by the supplier. In vitro packaging was performed by using commercial extracts purchased from Promega Biotec, Madison, Wis., following the directions of the supplier. Nuclease *Bal* 31 was used as previously described (21) to establish the relative order of cloned DNA fragments.

The characteristics of strain VW187 have been previously reported (34). The properties of the *E. coli* strains and plasmids used in this study are described in Table 1.

Transposition experiments. Plasmids pHoHo (Tn*3*-HoHo1) and pShe were used for transposition experiments carried out as described by Stachel et al. (30) on cosmid clones containing the O7 LPS genes. The target plasmids were transformed into HB101 (pHoHo and pShe), and the resultant strain was mated with HB101 (pRK2013) and the *polA* Nal^r recipient strain C2110. The triparental mating mixture was plated onto Luria agar plates containing nalidixic acid, tetracycline, and ampicillin to select for C2110 (target plasmid::transposon) exconjugants. Matings were performed by a plate mating method described by Bradley et al. (3).

Coagglutination. The presence of the O7 LPS antigen was determined by slide agglutination of antibody-coated staphylococci. O7 polyclonal rabbit antiserum (Centers for Disease Control, Atlanta, Ga.) was absorbed with protein A-containing *Staphylococcus aureus* Cowan 1 particles prepared as described by Kronval (13). A positive reaction was defined as the presence of visible agglutination within 3 min. Strong positive reactions were complete before the first minute. The coagglutination reagent detected up to 14 ng of purified O7 LPS.

Recombinant DNA methods. Recombinant plasmids were obtained by an alkaline lysis method (21). When needed, plasmid DNA was further purified by ultracentrifugation in cesium chloride-ethidium bromide density gradients (34). Total DNA from strain VW187 was isolated by a clear lysis method (9). Genomic DNA from strains belonging to different O types was obtained by the mini-scale procedure described by Owen and Borman (24). Electrophoresis of plasmid DNA was performed as previously described (34). Transformations were carried out by the calcium chloride method as described by Maniatis et al. (21). Cosmid cloning was performed as described elsewhere (35), using partially cleaved *Hin*dIII fragments of VW187 genomic DNA. After ligation to the vector pVK102, the DNA was in vitro packaged, and the lambda bacteriophage particles were transduced into *E. coli* K-12 strain LE392.

* Corresponding author.
† Present address: Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5C1, Canada.

TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype and relevant properties[a]	Reference
LE392	*hsdR supE44 supF lacY galK metB trpR*	21
HB101	*hsdR serA ara proA lacY galK rpsL xyl mtl supE*	21
C2110	*gyrA polA1 rha his*	16
MV104	VW187; *gal lacZ*	35
MV110	JC1552; *his*$^+$ *rfb* O7$^+$	This study
JC1552[b]	*leuB trp hisG argG metB lacY gal malA xyl mtl strA tonA tsx supE44*	17
SØ874[b]	*lacZ trp* Δ(*sbc*B-*rfb*) *upp rel rpsL*	22
MXR	Δ(*lac-pro*) *galE thi recA*	38
C94	O7:K1:H$^-$	1
OHSU1	O7:K1:H?	36
RS230	O1:K1	36
U41	O2:K1:H4	36
VW172	O6:K?	This laboratory
RS167	O16:K1:H6	1
RS218	O18:K1:H7	1
P7d	O68:K$^-$:H4	23
P10a	O71:K$^-$:H12	23
RS501	O75:K100	1
W28	O116:K?:H10	23
pVK102	Cosmid vector; Tc^r Km^r	10
pHP45Ω	Cloning vector; Ap^r Spc-Sm^r	28
pRK2013	RK2 derivative; Km^r *tra*$^+$	5, 6b
pHoHo	Tn*3*-HoHo1; Ap^r *lacIO lacZ*$^+$ *Y*$^+$*A*$^+$ *TnpA*	30
pShe	*TnpA*$^+$ Cm^r	30
pULB113	Tra$^+$ Ap^r Km^r Tc^r (Mu3A)	37
pJHCV31	O7$^+$ cosmid clone in pVK102; Tc^r	This study
pJHCV32	O7$^+$ cosmid clone in pVK102; Tc^r	This study
pJHCV31ΔSST	5-kb *Sst*I deletion from pJHCV31; Tc^r	This study
pJHCV32ΔSST	5-kb *Sst*I deletion from pJHCV32; Tc^r	This study
pJHCV64	15-kb *Bst*EII fragment from pJHCV32 cloned in pHP45Ω; O7$^+$ Ap^r	This study

[a] Abbreviations: Tc, tetracycline; Km, kanamycin; Ap, ampicillin; Spc, spectinomycin; Sm, streptomycin; Cm, chloramphenicol; *TnpA*, transposase.
[b] Strains obtained via B. Bachman, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

Hybridizations. The probe DNA fragments used in this study are described in the legend to Fig. 1 (probes 1, 2, and 3). DNA fragments were recovered in a strip of DE-81 filter paper (Whatman, Inc., Clifton, N.J.) inserted in the agarose gel just in front of the appropriate fragment. Upon continuing electrophoresis, the DNA fragment was retained in the filter paper and was further eluted by being washed with 1 M NaCl. The DNA fragment was concentrated by ethanol precipitation and labeled with [^{32}P]ATP (Amersham Corp., Arlington Heights, Ill.) by oligonucleotide synthesis (6). Southern blot hybridizations were carried out as described previously (34, 36).

LPS analysis. LPS from cell envelopes was extracted with hot phenol and was analyzed in silver-stained polyacrylamide gels (33). Total-cell envelopes obtained from 10-ml cultures were prepared as described previously (1), were suspended in 50 μl of water, and were treated with an equal volume of 90% phenol at 65°C for 15 min. The aqueous phase remaining after centrifugation at 16,000 × *g* was extracted once with ethyl ether, was mixed in a 1:1 ratio with a tracking dye solution (125 mM Tris hydrochloride [pH 6.8]–2% sodium dodecyl sulfate [SDS]–20% (vol/vol) glycerol–0.002% bromophenol blue–10% mercaptoethanol), and was boiled for 5 min before the gel was loaded. Polyacrylamide gels (14%; acrylamide-to-bisacrylamide ratio, 44:0.8) containing 4 M urea were electrophoresed at 20 mA of constant current (33). In some experiments, LPS material separated by SDS-polyacrylamide gel electrophoresis was subjected to a Western (immuno-) blot analysis as described by Towbin et al. (32). Blots were reacted with O7 antiserum and were developed with horseradish peroxidase-conjugated protein A as described elsewhere (2).

Phage sensitivity test for rough LPS. Sensitivity to the rough-specific bacteriophages Ffm, BR60, BR2, C21, and U3 tested as described elsewhere (4, 40) was used as an indication of the presence of rough LPS forms (lipid A-core oligosaccharide).

RESULTS

Cloning of the O7 LPS genes. Chromosomal DNA fragments from strain VW187 resulting from partial cleavage with *Hin*dIII were cloned into the low-copy-number vector pVK102 and were in vitro packaged into lambda phage particles as described in Material and Methods. Transductants were screened by coagglutination with an O7-specific polyclonal rabbit antiserum. Two positive-reacting colonies carrying the recombinant plasmids pJHCV31 and pJHCV32 were detected from a gene library of about 600 clones. Cells carrying pJHCV31 gave a weak coagglutination reaction (visible agglutination after 2 to 3 min) compared with that of cells carrying pJHCV32 (agglutination in less than 1 min, comparable to that of the wild-type strain VW187). None of these colonies reacted with staphylococcal particles prepared with O7 antiserum adsorbed with a Formalin-treated suspension of VW187 cells. Likewise, O7 antiserum previously adsorbed with one of these clones failed to agglutinate a VW187 cell suspension. Thus, the positive coagglutination phenotype expressed by the clones was specific for the O7 antigen.

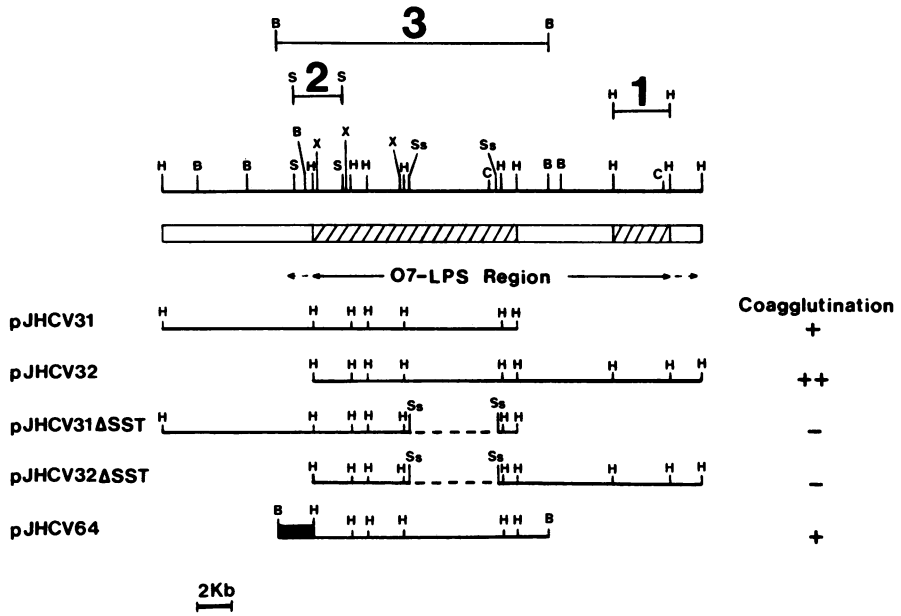

FIG. 1. Restriction endonuclease map of the O7 LPS region and adjacent sequences. The map was determined for the following enzymes: H, *Hin*dIII; B, *Bst*EII; X, *Xho*I; S, *Sal*I; Ss, *Sst*I; and C, *Cla*I. The restriction sites for *Cla*I are not completely mapped. The *Hin*dIII fragments in which insertions of transposon Tn*3*-HoHo1 resulted in an O7$^-$ phenotype by coagglutination (▨) and the *Hin*dIII fragments with either no transposon insertions or insertions not affecting the expression of O7 LPS (▭) are indicated. The approximate boundaries of the O7 LPS region are indicated by the arrows beneath the bars. Dotted arrows at the ends of the O7 LPS region indicate that the precise limit of this region is not yet determined. Inserted DNA (——) is indicated. pJHCV31 and pJHCV32 are the original cosmid clones giving a positive coagglutination test. The extent of the *Sst*I deletions in pJHCV31ΔSST and pJHCV32ΔSST (– – –) is shown. pJHCV64 was constructed by cloning a 14-kb *Bst*EII fragment from pJHCV32 into the *Bst*EII site of the streptomycin-spectinomycin adenyltransferase gene of pHP45Ω (8, 28). Thus, 1.8 kb of pVK102 vector DNA (■) were also included in the insert. The boundaries of DNA fragments used as radiolabeled probes (1, 2, and 3) are indicated. Probe 1 is a 3.3-kb *Hin*dIII fragment of pJHCV32; probe 2 is a 2.8-kb *Sal*I fragment from pJHCV31; and probe 3 is the 15-kb *Bst*EII insert of pJHCV64. The coagglutination reactions (strong [++], weak [+], or none [−]) are indicated.

A restriction endonuclease map of pJHCV31 and pJHCV32 was determined by digestion with various restriction endonucleases (Fig. 1). These experiments revealed that both plasmids had a common region consisting of *Hin*dIII fragments of 5.5, 2.3, 2.2, 0.85, and 0.7 kilobases (kb). To accurately establish the relative order of the *Hin*dIII fragments within the common region, a 5-kb *Sst*I fragment from pJHCV32 was deleted to generate a recombinant plasmid with a single *Sst*I recognition site. This plasmid, designated pJHCV32ΔSST, was digested with *Sst*I and treated with nuclease *Bal* 31. Samples were taken at various times and were further digested with *Hin*dIII. Restriction fragments were analyzed by gel electrophoresis, and the relative order of the *Hin*dIII fragments was deduced from the sequential disappearance of DNA fragments depending on the time of exposure to *Bal* 31 (Fig. 1; data not shown).

Hybridization experiments were carried out to assess whether the fragments flanking the right- and left-hand sides of the region common to pJHCV31 and pJHCV32 (Fig. 1) were present as such in the VW187 chromosome. A labeled 3.3-kb *Hin*dIII fragment of pJHCV32 (Fig. 1, probe 1) hybridized with the same 9.6-kb *Cla*I fragment in both pJHCV32 and the chromosome of strain VW187 (Fig. 2, lanes B and E). A similar experiment with a 2.8-kb *Sal*I fragment from pJHCV31 (Fig. 1, probe 2) demonstrated that the 8.5-kb *Hin*dIII fragment in pJHCV31 is also present in the VW187 chromosome (data not shown). Thus, no apparent rearrangements were found in the cloned DNA harboring the O7 LPS region.

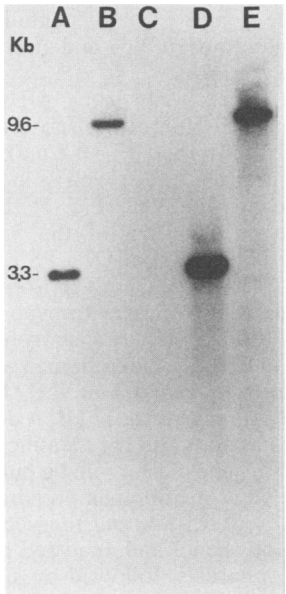

FIG. 2. Analysis of the regions flanking the O7 LPS gene sequences in strain VW187 by Southern blot hybridization of restriction endonuclease-cleaved DNA, using probe 1 (Fig. 1). Lanes: A and B, VW187 DNA; C, pJHCV31; D and E, pJHCV32. DNAs were digested with *Hin*dIII (lanes A, C, and D) or *Cla*I (lanes B and E).

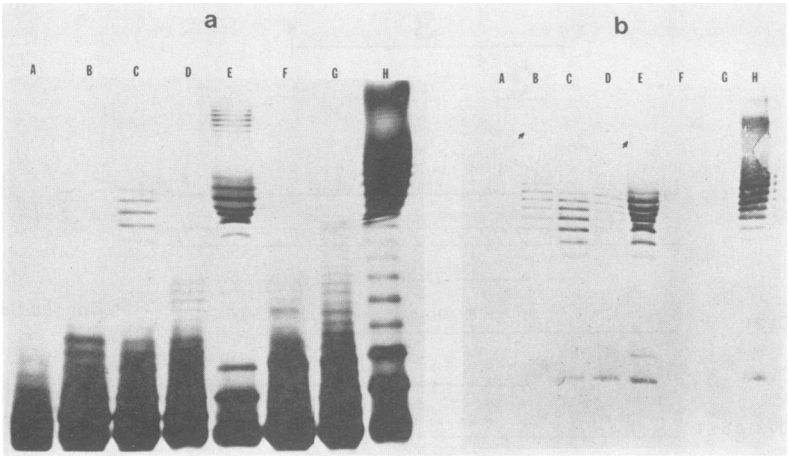

FIG. 3. Analysis of LPS by SDS-polyacrylamide gel electrophoresis and Western blotting. (a) Photograph of a 14% polyacrylamide gel stained with silver (32). Before electrophoresis, cell envelopes were extracted with hot phenol as described in Materials and Methods. Lanes: A, LE392; B, LE392 (pJHCV31); C, LE392 (pJHCV32); D, LE392 (pJHCV64); E, MV110; F, HB101 (pJHCV32ΔSST); G, C2110 (pJHCV32::Tn*3*-HoHo1-1); H, VW187. (b) Western blot of a gel similar to that in panel a, using O7-specific antibodies. Arrows (lanes B and E) point to a cluster of LPS bands barely seen in the photograph.

Localization of the O7 LPS region in the cloned DNA. The region of about 11 kb common to pJHCV31 and pJHCV32 was believed to harbor genes for the expression of the O7-positive-reacting phenotype. Cells carrying either pJHCV31ΔSST or pJHCV32ΔSST (Fig. 1) did not express O7 LPS as detected by coagglutination. This deletion eliminates most of the 5.5-kb *Hin*dIII fragment common to both plasmids (Fig. 1). However, since pJHCV31 conferred a weak positive coagglutination reaction, DNA sequences absent from this plasmid but present in pJHCV32 may be needed for the expression of O7 LPS. This hypothesis was confirmed by the subcloning of a 15-kb *Bst*EII fragment from pJHCV32 into the *Bst*EII site of pHP45Ω (28) which lies in the spectinomycin-streptomycin resistance gene of this plasmid (8). The resulting recombinant molecule, designated pJHCV64 (Table 1 and Fig. 1), contained the region of pJHCV32 common to pJHCV31 and also conferred a weak-positive coagglutination.

Transposition experiments using pJHCV32 as a target DNA showed that Tn*3*-HoHo1 transposon insertions lying in the 5.5-, 2.3-, 2.2-, 0.85-, and 0.7-kb *Hin*dIII fragments of the region common to pJHCV31 as well as in the 3.3-kb *Hin*dIII fragment unique to pJHCV32 (Fig. 1) resulted in an $O7^-$ phenotype. The participation of the 5.4-kb fragment of pJHCV32 in the expression of O7 LPS could not be determined, since no Tn*3*-HoHo1 transposon insertions in this fragment were detected. Similar transposition experiments using pJHCV31 showed that transposon insertions in the region common to pJHCV32 determined an $O7^-$ phenotype. These experiments indicated that the O7 LPS genes are clustered on a region of at least 17 kb. A detailed mapping of the transposon insertions and the identification of gene units among the O7 LPS gene cluster will be published elsewhere.

LPS analysis. Total membranes prepared from cells harboring pJHCV31, pJHCV32, and the vector pVK102 were extracted with hot phenol and analyzed by SDS-polyacrylamide gel electrophoresis, followed by silver staining (Fig. 3a, lanes A, B, and C). Only extracts from cells containing pJHCV31 and pJHCV32 showed a ladderlike banding pattern characteristic of O side chain material (Fig. 3a, lanes B and C). The O side chain bands reacted with an O7 antiserum as confirmed by a Western blot experiment (Fig. 3b). Western blotting proved to be more sensitive than silver staining, especially for detecting high-molecular-weight forms of O7 LPS expressed by the recombinant plasmids. The results therefore suggested that pJHCV31 and pJHCV32 possessed information for synthesizing an O7 polysaccharide that is covalently linked to the lipid A-core oligosaccharide of *E. coli* K-12. Although the O7 LPS bands determined by pJHCV31 were of a higher molecular weight than those encoded by pJHCV32, the amount of O7 LPS determined by the former plasmid was lower than that mediated by pJHCV32. The differences in the expression of O7 LPS may account for the weak coagglutination reaction of cells carrying pJHCV31 (see above). In addition, the high-molecular-weight LPS forms found in cells carrying pJHCV31 may indicate the participation of sequences from the 8.5-kb *Hin*dIII fragment in the expression of O7 LPS. The contribution of the *E. coli* K-12 chromosomal *rfb* genes in the formation of the O7 oligosaccharide unit was ruled out since pJHCV31 and pJHCV32 also expressed O7 LPS in *E. coli* K-12 strain SØ874 (Table 1), which has a chromosomal deletion eliminating the *rfb* region (data not shown). Moreover, envelope extracts from cells containing pJHCV32Δ SST and pJHCV32 with a Tn*3*-HoHo1 insertion in the 5.5-kb *Hin*dIII fragment did not express O7 LPS (Fig. 3a and b, lanes F and G).

Silver-stained gels and Western blot experiments showed that both the average length and the overall amount of O7 LPS molecules in strains of *E. coli* K-12 carrying pJHCV31 and pJHCV32 were lower than those of strain VW187 (Fig. 3a and b, lanes B, C, and H). However, higher-molecular-weight forms of O7 LPS comparable to those present in strain VW187 were found in the *E. coli* K-12-VW187 hybrid strain MV110 (Table 1; Fig. 3a and b, lanes E). This strain resulted from conjugation and further recombination of VW187 chromosomal markers ($O7^+$ His^+) into *E. coli* K-12 strain JC1552 ($O7^-$ *his*; see below). Thus, it is likely that other DNA sequences from VW187 not present in pJHCV31 and pJHCV32 are necessary for a full expression of the O7 LPS gene cluster in *E. coli* K-12.

The lower O7 LPS expression in *E. coli* K-12 suggested that in this strain only a fraction of lipid A-core molecules was substituted with O7 oligosaccharide repeating units. The

TABLE 2. Lysis by bacteriophages

Strain[a]	Recombinant plasmid	Bacteriophage[b]				
		Ffm	C21	BR60	BR2	U3
LE392	None	+	+	+	+	+
LE392	pJHCV31	+	+	+	+	+
LE392	pJHCV32	+	+	+	+	+
MV110	None	+	+	+	+	+
VW187	None	−	−	−	−	−
MV104	None	+	+	+	+	−

[a] See Table 1 for strain and plasmid descriptions.
[b] Sensitivity to bacteriophages was tested as described previously (40). +, Lysis halo; −, no lysis. Bacteriophages Ffm, C21, BR60, and BR2 recognize lipid A-core of various chemotypes, whereas bacteriophage U3 is specific for the *E. coli* K-12 lipid A-core.

use of rough-specific phages confirmed this suggestion. Strain LE392 containing pJHCV31 and pJHCV32 remained sensitive to the rough-specific phages C21, Ffm, BR60, and BR2 (Table 2), as did strain MV110. All of these strains were sensitive to phage U3, which recognizes only the *E. coli* K-12 lipid A-core chemotype. In contrast, the wild-type strain VW187 was not lysed by any of these phages. The failure to lyse VW187 was not due to restriction of phage DNA since strain MV104, a *gal* mutant of VW187 expressing only a rough LPS, was lysed by all rough-specific phages except U3. Therefore, these experiments indicated that *E. coli* K-12 strains harboring O7 LPS genes possess lipid A-core molecules lacking covalently attached O7 polysaccharides.

Localization of the O7 LPS genes near the *his* locus. The O side chain genes in certain *E. coli*, *Salmonella*, and *Shigella* strains are located near the *his* locus at 42 min of the chromosome gene linkage map (20, 23). To demonstrate whether the O7 LPS genes are also located near the *his* region, chromosomal markers from strain VW187 were mobilized to *E. coli* K-12. Strain VW187 was first mated with strain MXR containing the RP4-mini-Mu derivative pULB113 (Table 1). This plasmid has the properties of being able to integrate at random into the chromosome and to mobilize chromosomal markers by conjugation (37). The resultant exconjugant, VW187 (pULB113), was then mated with strain JC1552, a multiauxotrophic strain of *E. coli* K-12 with a mutation in the *his* operon. Although only a few his^+ exconjugants were obtained, all of them showed a positive coagglutination with the O7 antiserum. One of these exconjugants, designated strain MV110, did not possess characteristics of VW187 other than the His^+ and $O7^+$ phenotypes. However, strain MV110 did not express wild-type levels of O7 antigen as assessed by SDS-polyacrylamide gel electrophoresis and rough-specific phages (Fig. 3a and b, lanes E, and Table 2). A hybridization experiment with an O7-specific probe fragment (Fig. 1, probe 3) carried out on *Hin*dIII-cleaved genomic DNA from one of these exconjugants (Table 1, strain MV110) and from strain VW187 resulted in the detection of the same restriction fragments in both cases (data not shown). No hybridization with this probe was detected in JC1552 DNA. These experiments demonstrated that, as a result of conjugation and further recombination of chromosomal markers, the *E. coli* K-12 *his-rfb* region was replaced by the VW187 his^+ $O7^+$ gene sequences and, therefore, the O7 LPS genes must be linked to the *his* region.

Absence of O7 LPS gene sequences in other *E. coli* O types. The existence of homologies at the DNA level between the O7 LPS genes and O side chain genes from other *E. coli* strains was examined by DNA-DNA hybridizations. Southern blots of *Hin*dIII-cleaved genomic DNAs from *E. coli* clinical strains belonging to the types O1, O2, O6, O16, O18, O68, O71, O75, and O116 and from *E. coli* K-12 were hybridized with an O7-specific probe consisting of the radiolabeled 15-kb *Bst*EII insert from pJHCV64 (Fig. 1, probe 3). The probe hybridized to the expected DNA fragments from pJHCV32 (Fig. 4, lane B) and from the three members of the O7 type (Fig. 4, lanes C, D, and E). Since the probe fragment contained a portion of the *cos* site region from the lambda phage (see legend to Fig. 1) (10), a positive hybridization was detected with the 23-kb *Hin*dIII fragments from both lambda phage and pVK102 (Fig. 4, lanes A and B). No significant homologies were detected between the probe and the chromosomal DNA of the *E. coli* strains with O types other than O7 (Fig. 4, lanes F through O), demonstrating that these *E. coli* strains do not contain sequences in common with the O7-specific probe fragment.

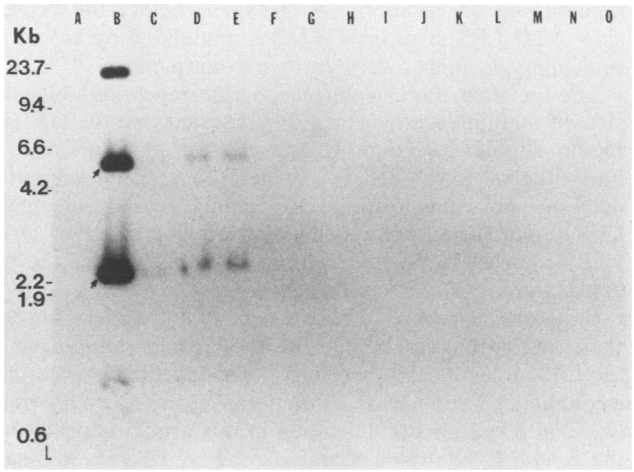

FIG. 4. Southern blot hybridization of chromosomal DNAs with the O7-specific probe. Genomic DNAs were cleaved with *Hin*dIII, transferred to nitrocellulose, and hybridized by using the 15-kb *Bst*EII insert (Fig. 1, probe 3) as a radiolabeled O7-specific probe. Lanes: A, *Hin*dIII-cleaved lambda phage DNA; B, pJHCV32; C, C94 (O7:K1); D, VW187 (O7:K1); E, OHSU1 (O7:K1); F, LE392 (*E. coli* K-12); G, RS230 (O1); H, U41 (O2); I, VW172 (O6); J, RS167 (O16); K, RS218 (O18); L, P7d (O68); M, P10a (O71); N, RS501 (O75); O, W28 (O116). Arrows (lane A) point to hybridizing fragments of 5.4 and 5.5 kb and 2.3 and 2.2 kb. Lower-molecular-weight fragments of 0.85 and 0.7 kb which hybridize with the probe in lanes C, D, and E are barely visible in the photograph.

DISCUSSION

The O7 LPS gene determinants from a septicemic strain of *E. coli* O7:K1 were cloned and expressed in *E. coli* K-12. In addition, O7 LPS *rfb* genes were mapped near the *his* region of the chromosome. This is in agreement with previous studies showing that the genes which control the biosynthesis of polysaccharide components of LPS in *E. coli* are usually located on the bacterial chromosome (20, 23). However, it has been recently demonstrated that in the case of *Shigella dysenteriae* a small plasmid encodes the first biosynthetic enzyme for the O side chain LPS (31, 39), whereas in *Shigella sonnei* and in a strain of *E. coli* O111 the complete *rfb* gene cluster is plasmid mediated (11, 29). We do not have any evidence of a plasmid localization of genes involved in the O7 LPS biosynthesis. The curing of a 43-kb plasmid, the largest extrachromosomal element possessed by this isolate (34), did not affect O7 LPS expression (M. A. Valvano,

unpublished observations). However, whether the expression of O7 LPS in strain VW187 is modulated by any of its remaining plasmids awaits further experiments.

The fact that the O7 oligosaccharide repeating unit consists of five different sugars (19) suggested that the O7 LPS region should be complex and consist of a number of biosynthetic and regulatory genes. Restriction mapping, deletion, and transposition experiments carried out on the $O7^+$ recombinant plasmids defined a region of about 17 kb which appears to be essential but not sufficient for the full expression of O7 LPS in *E. coli* K-12.

Strains of *E. coli* K-12 have a defective O-antigen biosynthesis region but can supply the lipid A-core component of the LPS molecule and therefore can support the synthesis of a complete LPS molecule if functional *rfb* genes are provided (20, 23). Experiments described in this article indicate that the *E. coli* K-12 strains containing the O7 LPS recombinant plasmids pJHCV31 and pJHCV32 produced less O7 LPS material, and lower in molecular weight, than did the wild-type strain VW187. The fact that the O7 antigen was covalently attached to the lipid A-core is suggested by the positive staining with silver (14). However, *E. coli* K-12 containing either pJHCV31 or pJHCV32 remained sensitive to rough-specific phages regardless of the production of silver-stained O7 LPS material, whereas the wild-type strain was phage resistant. The *E. coli* K-12-VW187 hybrid strain MV110 was also sensitive to rough-specific phages even though it expressed more O7 side chain than did cells carrying pJHCV31 or pJHCV32. Our findings therefore suggest that the low amounts of O7 LPS in cells carrying either pJHCV31 or pJHCV32 and in strain MV110 could be due to a partial defect in the transfer of O7 polysaccharide to the *E. coli* K-12 lipid A-core. This possibility is sustained by previous work demonstrating that in *E. coli* K-12 only a portion of the core oligosaccharide is completed (27). Also, other DNA regions in strain VW187 not present in the cloned DNA may be playing a regulatory role in determining the amount of O7 antigen synthesized and linked to the lipid A-core.

The chemical composition, structure, and antigenicity of O side chains vary widely among *E. coli* and other enteric bacteria (18, 23). Selective pressures presumably led to a tremendous diversification in the O side chain structure. The molecular nature of this variation remains to be explored. Hybridization experiments carried out in this study demonstrated that at least a large portion of the O7 LPS region does not show any detectable homology at the DNA level with other O-type genes. These results suggest that the O7 LPS genes, and possibly other O-type genes, are unique and may have arisen as a result of genetic rearrangements which occurred during the evolution of the *rfb* region. However, it is important to consider that the strains examined in this work possess O types that are chemically different from the O7 LPS (18, 23). Therefore, it may be possible to find homologies among LPS genes of bacterial strains expressing O side chains that are chemically related. This conclusion is supported by recent evidence from other investigators showing that LPS *rfb* genes from two *Salmonella* subspecies possess both homologous and nonhomologous DNA regions (38). Experiments are under way to compare the O7 LPS genes with the corresponding genes from other *E. coli* and *Shigella* sp. strains expressing O side chains chemically and immunologically related to the O7 polysaccharide.

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