In *Escherichia coli* F632, the 14-kilobase pair chromosomal region located between *wacC* (formerly *rfac*) and *waaA* (*kdtA*) contains genes encoding enzymes required for the synthesis of the type R2 core oligosaccharide portion of lipopolysaccharide. Ten of the 13 open reading frames encode predicted products sharing greater than 90% identity with homologs in *E. coli* K-12. However, the products of *waaK* (*rfak*) and *waaL* (*rfal*) each resemble homologs in *Salmonella enterica* serovar *Typhimurium* but share little similarity with *E. coli* K-12. The F632 WaaK and WaaL proteins therefore define differences between the type R2 and K-12 outer core oligosaccharides of *E. coli* lipopolysaccharides. Based on the chemical structure of the core oligosaccharide of an *E. coli* F632 *waaK::aacC1* mutant strain, and in vitro glycosyltransferase analyses, *waaK* encodes UDP-N-acetylmuramylpentapeptide glucosaminyltransferase, and *waaL* encodes N-acetylmuramylpentapeptide glucosaminyltransferase. The WaaK enzyme adds a terminal GlcNAc side chain substituent that is crucial for the recognition of core oligosaccharide acceptor by the O-poly saccharide ligase, WaaL. Results of supplementation analyses of *E. coli* K-12 and F632 *waaL* mutants suggest that structural differences between the WaaL proteins play a role in recognition of, and interaction with, terminal lipopolysaccharide core moieties.

Lipopolysaccharides (LPS) are major and characteristic components of the outer membrane of Gram-negative bacteria. The hydrophobic lipid component (lipid A) anchors the LPS molecule in the outer membrane. Lipid A is linked to a core oligosaccharide (core OS) of 10–15 sugars; the core OS is often luxuriently substituted. The resulting basic structure is known as rough or R-LPS. In the *Enterobacteriaceae*, R-LPS is capped by an O antigen side chain polysaccharide (O-PS) to form LPS molecules termed smooth (or S-LPS). In contrast, some organisms, like *Neisseria meningitidis*, lack O-PS but modify their R-LPS by addition of a few glycosyl residues to produce lipo-oligosaccharide. In the *Enterobacteriaceae*, the core OS is divided into two structural regions, an inner core containing Kdo and heptose and an outer core region consisting primarily of hexose and acetamido sugars. Whereas the inner core is highly conserved among members of the *Enterobacteriaceae*, the outer core region exhibits variation in its components and structure. Indeed, although there is only one wild-type core structure currently described in *Salmonella* spp. (Ra core), there are five different core OS structures in *Escherichia coli* (designated K-12, R1, R2, R3, and R4) which are differentiated based on their outer core OS structures. The structures of the outer core OSs of *Salmonella enterica*, *E. coli* K-12, and *E. coli* R2 are shown in Fig. 1A.

Lipid A-core and O-PS are formed by independent assembly pathways (1–3). The core OS biosynthesis region of the chromosome (formerly known as the *rfa* region) contains genes that define unique core OS structures. Many of the genes at this locus code for glycosyltransferases which sequentially elongate the core OS on a lipid A acceptor. The chromosomal core OS biosynthesis region of *E. coli* K-12 has been entirely sequenced, and the majority of the equivalent region has been completed in *S. enterica* (Fig. 1B). Most of the known core OS biosynthesis genes in *S. enterica* have predicted products that are highly similar (greater than 70% total similarity) to *E. coli* K-12 counterparts. Striking exceptions are WaaK and WaaL, where the similarity is less than 35% (4). The WaaL protein is the only gene product known to be involved in the ligation of pre-assembled O-PS to lipid A-core. This occurs at the periplasmic face of the plasma membrane, prior to translocation of completed S-LPS to the outer membrane (reviewed in 1). WaaL mutants of both *E. coli* K-12 and *S. enterica* are unable to "cap" the lipid A-core molecule with an O-PS. The WaaL enzyme of *E. coli* K-12 has relaxed specificity for the polymer it attaches to lipid A-core since it can effectively ligate a number of "native" *E. coli* polymers, as well as an ever increasing range of O-PS structures resulting from expression of cloned O-PS-biosynthesis genes in *E. coli* K-12 (1). From the limited available data, it appears that the *S. enterica* WaaL protein shows similar relaxed specificity for polymer structure. Ligase enzymes from different bacteria are therefore expected to share a common

---

`* This work was supported in part by funding awarded (to C. W.) by the Canadian Bacterial Diseases Network and by the Natural Sciences and Engineering Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF026386 and AF019375.

† Recipient of a Natural Sciences and Engineering Research Council postdoctoral fellowship.

‡ To whom correspondence should be addressed. Tel.: 519-824-4120 (ext. 3478); Fax: 519-837-1802; E-mail: cwhitfie@uoguelph.ca.

§ The abbreviations used are: LPS, lipopolysaccharide; PAGE, polyacrylamide gel electrophoresis; S-LPS, smooth LPS; R-LPS, rough LPS; Tricine, N,N,N′,N′-tetraethyltris(carboxymethyl)ethyleneurea; kb, kilobase pairs; Kdo, 3-deoxy-d-manno-oct-2ulosonic acid; O-PS, O-poly saccharide; core OS, core oligosaccharide; PCR, polymerase chain reaction; HCA, hydrophobic cluster analysis.

Since most data supports the presence of a single core structure in the genus *Salmonella* and the majority of genetic and biochemical data in this area is confined to *Salmonella enterica* serovar *Typhimurium*, this bacterium will be simply referred to as *S. enterica* in this communication.
FIG. 1. Structure of the outer core OS of E. coli K-12, E. coli R2, and S. enterica and organization of their core OS biosynthetic clusters. A, structure of the outer core OSs from the LPSs of E. coli K-12, E. coli R2, and S. enterica. Genetic determinants involved in their biosynthesis are also indicated. All sugars are in the pyranose configuration and the linkages are α, unless otherwise indicated. B, maps of the sequenced regions of the waa cluster from the chromosomes of S. enterica, E. coli K-12, and E. coli R2. Numbers indicate percent similarity and identity at both the amino acid and nucleotide levels for respective homologs. The waa* nomenclature is described elsewhere (http://www.angis-s.une.edu.au/E.coli.html, Ref. 43). Genes involved in the synthesis of the outer core OS are highlighted in white, and the waaL gene, which is involved in ligation of O-PS to lipid A-core, is highlighted in gray. The nucleotide sequence from waaC to waaA of the E. coli F632 chromosome has been deposited in the GenBank™ data base under accession number AF019375, and the nucleotide sequence from waaY to waaA of the S. enterica chromosome has been deposited in the GenBank™ data base under accession number AF026386.

mechanism of action. Although there is little similarity between the primary sequence of the WaaL homologs of E. coli K-12 and S. enterica, both are integral membrane proteins and have similar hydropathy profiles (4).

Ligation is a crucial step in the assembly of S-LPS. Since the O-PSs of pathogenic bacteria are usually required for resistance to complement-mediated killing (5), the ligation step is important for survival in the host and could potentially be exploited for novel therapeutic approaches. However, the mechanism of ligation is unknown. Differences in WaaL sequences of E. coli K-12 and S. enterica most likely reflect the varying structures in the outer core OSs (see Fig. 1A) which serve as acceptors for O-PS, but the structural requirements for a functional core OS acceptor have not been addressed in a systematic manner. Attempts to relate structure and function in WaaL homologs from E. coli K-12 and S. enterica are hampered by differences in both backbone glycan sequence as well as side chain substitutions in their respective core OSs. The E. coli R2 core OS has a backbone identical to E. coli K-12 but contains a terminal α1,2-GlcpNac side branch, as is found in S. enterica (Fig. 1A). Analysis of WaaL activity in this strain therefore allows distinction between structural requirements for ligation imposed by features of the core OS backbone and terminal side branch substitutions.

To resolve these ambiguities, the waaK and waaL genes were characterized in E. coli F632, a prototype strain with an E. coli R2 core OS. Structural and biochemical analyses of defined insertions in the E. coli R2 chromosomal genes, together with complementation experiments using single open reading frames and E. coli R2, K-12 and S. enterica core OS acceptors were used to precisely define the effects of the terminal GlcNac side branch of the E. coli R2 core OS on ligation activity.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The bacterial strains and plasmids used in this study are listed in Table I. The R2 prototype strain used in this study, F632, is an O-PS-deficient derivative of E. coli O100, and although it does not produce an O-PS, it does contain a complete core OS (this study and Refs. 8 and 9).

Media and Growth Conditions—Bacterial strains were routinely grown in Luria-Bertani (LB) broth (10) at 37 °C, unless otherwise stated. Growth medium was supplemented with ampicillin (100 µg/ml), chloramphenicol (30 µg/ml), gentamicin (15 µg/ml), kanamycin (30 µg/ml), or tetracycline (10 µg/ml) as necessary. L-Arabinose was used at a final concentration of 0.02% for growth and induction of strains containing pBAD18 derivatives.

DNA Methods—Restriction endonuclease digestion and ligation was performed essentially as described by Sambrook et al. (11). Restriction enzymes were purchased from either Life Technologies, Inc. (Burlington, Ontario), New England Biolabs (Mississauga, Ontario), or Boehringer Mannheim (Laval, Quebec). Plasmids were introduced into E. coli strains by using CaCl2-competent cells (11) or by electroporation using conditions described elsewhere (12) and a Gene Pulser from Bio-Rad (Mississauga, Ontario). Chromosomal DNA isolation was performed using the Qiagen genomic DNA isolation kit, and plasmid DNA was prepared using QIAprep plasmid spin columns (Qiagen Inc., Santa
**E. coli R2 LPS Core Biosynthesis**

| Strain or plasmid | Relevant properties | Source or reference |
|-------------------|---------------------|---------------------|
| E. coli DH5α      | K-12 F- 880d supE44 ΔlacZYA-argF U196 endA1 hsdR17 (rK-12 mK-12) recA1 | Novagen |
| BL21 (DE3)        | E. coli B F− hsdS (rK-12 mK-12) gal dcm ompT (DE3) |  |
| SM10apir          | K-12 apir recA::RFP–2-Tc::Mu Km− |  |
| AB1133            | K-12 thr-1 leuB6 lacY1 supE44 rmd1 D1 thi-1 mtlA1 tra-14 galK2 xyl-5 mtl-1 |  |
| CS180             | P1700 of P. Reeves, derived from AB1133; his leu proA argT his thi galK lacY trpE |  |
| CS2529            | waaU::Kan derivative of CS180 |  |
| CS2334            | waaL::TuphoA derivative of CS180 |  |
| F632              | E. coli R2; R-LPS derivative of O100-K7 (B); H2 |  |
| CWG300            | waaK::aacC1 derivative of F632; Gm′ | This study |
| CWG302            | waaL::aacC1 derivative of F632; Gm′ | This study |
| S. enterica LT2    | waa−; S-LPS |  |
| SL3770            | waaK953; R-LPS |  |
| SL3749            | waaLA464; R-LPS |  |
| Plasmids          |                     |  |
| pBluescriptII SK(+) | Multicyclic phagemid cloning vector derived from pUC19; Ap′ | Stratagene |
| pBC SK(+ )        | Multicyclic phagemid cloning vector derived from pUC19; Cm′ | Stratagene |
| pUCGM             |                             |  |
| pCVD442           | oriR6K mobRP4 strB blasM, positive-selection suicide vector; Ap′; Cm′; Km′ |  |
| pMAK705           | pUC19-pMAK700 hybrid suicide vector containing the temperature-sensitive pSC101 replicon; Cm′ |  |
| pET30α (-)        | T7-Based expression vector; Km′ | Novagen |
| pBAD18            | expression vector containing the pBAD promoter; Ap′ |  |
| pWQ3              | pKK404-derivative containing O-P-S biosynthesis cluster of K. pneumoniae O1; Tc′ |  |
| pWQ900            | pBluescript II SK (+)-derivative which carries a 3.3-kb PCR-amplified fragment of F632 DNA spanning the waaK and waaL coding regions; Ap′ | This study |
| pWQ901            | pBAD18-derivative carrying waaK from F632; Ap′ | This study |
| pWQ902            | pBAD18-derivative carrying waaL from F632; Ap′ | This study |

*Salmonella Genetic Stock Center; Ap′, ampicillin-resistant; Cm′, chloramphenicol-resistant; Gm′, gentamicin-resistant; Km′, kanamycin-resistant; Tc′, tetracycline-resistant.*

Clara, CA). Where necessary, DNA fragments were isolated from agarose gels using the GeneClean kit from Bio/Can Scientific (Mississauga, Ontario).

**PCR and Sequencing Techniques—Oligonucleotides were synthesized using the Perkin-Elmer 394 DNA synthesizer, and sequencing was performed using an ABI 377 DNA sequencing apparatus (Perkin-Elmer) at the Guelph Molecular Supercentre (University of Guelph). PCR was performed using a GeneAmp PCR System 2400 from Perkin-Elmer. The “expand high-fidelity enzyme mix” (Boehringer Mannheim) was used as the polymerase enzyme in PCR reactions where products were greater than 5 kb. For product sizes of less than 5 kb, Pwo I DNA polymerase (Boehringer Mannheim) was used. PCR amplification of the 14-kb fragment flanked by the waaC and waaA genes was performed as follows: one initial cycle at 94 °C for 1 min; 20 cycles at 94 °C for 15 s and 68 °C for 12 min; 16 auto cycles at 94 °C for 15 s and 68 °C for 12 min, with an auto-extension of 68 °C for 15 s per cycle; a final cycle at 72 °C for 10 min. The oligonucleotide primers were based upon similar regions of sequence between E. coli K-12 and S. enterica in the waaC and waaA genes and are as follows: (i) forward primer, 5'-ACGTTGGCCCGCACTCAGTGA-3'; and (ii) complementary reverse primer, 5'-TTCGGTGGCAGGTAAGGTTC-3'. PCR products were purified using the QIAquick PCR purification kit from Qiagen. To ensure error-free sequencing, the sequence of each of the DNA strands was determined from the product of separate PCR runs. In the rare instances where a mismatch in sequence between strands occurred, a small region surrounding the mismatch was reamplified and resequenced.

**In Vitro Mutagenesis and Gene Replacement—** The E. coli F632 waaK gene was mutated in vitro by insertion of a gentamicin-resistance cassette (the aacC1 gene from Tn1696). The cassette was isolated on a 835-bp SacI fragment from plasmid pUCGM, blunt-ended with T4 DNA polymerase, and inserted into the unique EcoRI site in the waaK coding region of plasmid pWQ900 (Fig. 2). The waaK::aacC1 gene was then recovered on a 1.8-kb SacI fragment which was inserted into the Smal site of the suicide delivery vector pCVD442 (13). Plasmid pCVD442 carrying the waaK::aacC1 gene was maintained in the mobilizing strain SM10apir and transferred to E. coli F632 by conjugation. E. coli F632 waaK::aacC1 was obtained by sucrose selection in the absence of NaCl at 37 °C. Resolving colonies were tested for gentamicin resistance and ampicillin sensitivity. The presence of the waaK::aacC1 mutation was confirmed by Southern hybridization and PCR, followed by sequencing the junction sites of waaK::aacC1 on the amplified fragment. The E. coli F632 waaL gene was mutated in vitro by replacement of an internal 1.2-kb HpaI-MfeI fragment from the waaL coding region.

---

**FIG. 2. Physical map of the PCR-amplified fragment present within pWQ900.** The coding regions of waaC, L, K, and Z are indicated. The top portion of the figure indicates the region of DNA from pWQ900 that was used to make the chromosomal insertion in waaK and deletion in waaL to derive strains CWG300 and CWG302, respectively. The bottom portion of the figure indicates the region of DNA that was PCR-amplified to clone the waaK and waaL genes downstream of the P23i promoter in plasmid pBAD18 to generate plasmids pWQ901 and pWQ902, respectively. The small arrows designate the direction of transcription from P23i.
of pWQ900 (Fig. 2) with the gentamicin resistance cassette present on a Smal fragment from plasmid pUCGM. This essentially removes the complete waal coding region. The waal::aacC1 gene was recovered on a 2.5-kb BamHI-BstXI fragment which was blunt-ended with Klenow enzyme and T4 DNA polymerase and inserted into the unique EcoRI site of the transient expression vector pMtk705 (14). Each bacterial cell was transformed with pMtk705 carrying the waal::aacC1 gene, and chromosomal gene replacement was carried out by a procedure described elsewhere (15). The presence of the waal::aacC1 mutation in E. coli CG302 was confirmed by sequencing the junctions of waal::aacC1 in an amplified PCR fragment.

Computer Analysis—Sequence data were edited and analyzed using AssemblyLIGN and MacVector software (International Biotechniques Inc., New Haven, CT). Hydrophilicity plots of predicted amino acid sequences were performed using the MacVector software package and the method of Kyte-Doolittle, with a hydrophilicity window of 7 and an amphipathicity window of 11. Homology searches of nucleotide and amino acid sequences in the National Center for Biotechnology Information data bases were done with the BLAST (basic local alignment search tool) server analysis program (16). Pairwise nucleotide sequence alignments and percentage identity scores were obtained using the NALIGN program of the PC/GENE software package (IntelliGenetics Inc, Mountain View, CA) with an open gap cost of 25 and a unit gap cost of 5. Pairwise protein alignments and percentage identity and similarity scores obtained using the PHALIGN program of PC/GENE with an open gap cost of 5 and a unit gap cost of 5. Multiple sequence alignments were performed using CLUSTALX (version 1.62b). Protein secondary structure was predicted using the GARNIR and GGBSM programs present in the PC/GENE software package and by hydrophobic cluster analysis (HCA) (using the HCA plot program (Doriane Informatique, Le Chesnay, France).

Lipopolysaccharide Analysis by SDS-PAGE—Small scale LPS preparations were made from SDS-proteinase K whole cell lysates by the method of Hitchcock and Brown (17). Large scale preparations used the hot phenol/water extraction as described elsewhere (18). LPS was separated on 10–20% gradient SDS-Tricine polyacrylamide gels that were obtained from Novex (San Diego, CA). Polyacrylamide gel electrophoresis (PAGE) conditions were those recommended by the manufacturer. Silver staining (19) and Western immunoblotting procedures have been described (15), as has production of polyclonal rabbit anti-n-galactan I serum (20). Throughout this study, LPS from an equivalent number of cells was loaded in each gel lane.

Generation of Core Oligosaccharides—Water-insoluble LPSs were obtained by hot water/phenol extraction of E. coli F632 and CG302 cells, and treated with 1% acetic acid at 100 °C to cleave the acid-labile ketosidic linkage between the core and lipid A. The water-insoluble lipid A was isolated from the hydrolysate as a pellet by hot phenol/water extraction as described elsewhere (18). LPS was obtained from 500 ml of log phase E. coli F632 (R2 core prototype) was determined. In E. coli K-12 and S. enterica, the waac and waao genes encode the heptosyltransferase I and the bifunctional Kdo transferase, respectively, for inner core biosynthesis (3). Similarities in the waac and waao genes (and gene products) between E. coli K-12 and S. enterica and preliminary Southern hybridization experiments (data not shown) suggested that these genes would likely be conserved in other E. coli core types. This predicted conservation was used to design PCR primers to amplify the region containing the outer core OS biosynthesis genes from E. coli F632. The complete nucleotide sequence of the resulting 14-kb PCR amplification fragment was determined, revealing a general organization typical of those seen in E. coli K-12 and S. enterica (Fig. 1B). The organization and function of the core OS biosynthesis regions in S. enterica and E. coli K-12 have been reviewed previously (2). However, the sequence information for S. enterica was incomplete and, in some regions contained errors, effectively limiting comparisons with the region from E. coli K-12. These problems were resolved by sequencing PCR amplification products spanning gaps in S. enterica sequences and resequencing regions where some ambiguities remained. The structures of the completed regions from the three bacteria are shown in Fig. 1B as are the sequence relationships between the genes and their predicted gene products.

Although the functions of some outer core OS biosynthesis enzymes have been established in biochemical analyses, others are inferred from structure of mutant core OSs resulting from defects in various genes (for reviews see Refs. 2 and 3). The relationships among predicted polypeptides representing the core OS backbone glycosyltransferases in S. enterica, E. coli K-12, and E. coli R2 are consistent with the structures of their respective core OSs.

All three core types have a G1p-a(1–3)-Hepp linkage defining the junction between the inner and outer core OS. The waac gene encodes a UDP-glucose:heptosyl lipid A polysaccharide a1,3-glycosyltransferase (GlcI transferase) in S. enterica (27), and the OS chemical structure in a waac mutant is consistent with a similar activity in E. coli K-12 (28). The S. enterica waac mutant is complemented by the cloned genes from both S. enterica (29) and E. coli K-12 (30). The Waac predicted proteins of S. enterica and E. coli K-12 and F632 share 85.8–96.0% identity (Fig. 1B). The three known Waac proteins all contain a motif characteristic of one family of α-glycosyltrans-
ferases (31). The motif comprises two invariant glutamic acid residues in the signature sequence E(X₈)E, located in a region of similar secondary structure as defined by hydrophobic cluster analysis (HCA). This sequence in all three WaaG proteins is E₂⁸⁶AAGIVLLE₂⁹⁹. These data, together with similar genetic organization are consistent with the assignment of the waaG gene in E. coli F632.

All three core types have an α,1,6-linked Galp side branch on the GlcI residue. Structural determination of mutant LPSs, enzyme assays, and genetic complementation experiments all identify waaB as the structural gene for the UDP-galactose: (glucosyl)LPS α,1,6-galactosyltransferase in S. enterica (29, 32). The transferase involved, WaaB, is highly conserved. The E. coli F632 WaaB protein shares 63.2% identity with the E. coli K-12 protein (92.5% identity). The S. enterica waaI and waaJ genes (33) identified smaller open reading frames than the waaO and waaR genes (33). On resequencing in the current study, the predicted WaaI and WaaJ proteins are found to be of comparable size to their E. coli and S. enterica waaO and waaR counterparts. The WaaO, -R, -I, and -J transferases all lack the α-glycosyltransferase motif typical of WaaB and WaaG. However, BLASTP searches of the data bases identify consensus features in these proteins which are found in a variety of other prokaryotic α-glycosyltransferases and thus define a new family of α-glycosyltransferases (Fig. 3 and Table III) which is discussed below.

New Family of α-Glycosyltransferases: the WaaIJ Family—WaaI, and to a lesser extent WaaJ, of S. enterica have been established as the HexII and HexIII glycosyltransferases, respectively, involved in assembly of the outer core OS portion of the LPS molecule. The HexII and HexIII glycosyltransferases of E. coli K-12, WaaO, and WaaR have been identified, although direct biochemical evidence conclusively identifying their functions is currently limited. These proteins share four highly conserved regions of primary sequence (labeled I, II, III, and IV) which are highlighted in Fig. 3. BLASTP searches of the data bases identify consensus features in these proteins which are found in a variety of other prokaryotic α-glycosyltransferases and thus define a new family of α-glycosyltransferases (Fig. 3 and Table III) which is discussed below.

Characterization of the E. coli R2 waaK Gene—The E. coli R2

| HexII transferases | WaaI  | S. enterica  | 127 VLYLDADIAAKSGSLQEL | 181 YFNAFGLILN | 220 DDD | 263 FHHTYGPRTKWH |
|--------------------|-------|--------------|------------------------|---------------|---------|------------------|
| WaaO               | E. coli K-12 | 127 VLYLDADIIQCTTEFLP | 181 YFNSGFLILN | 220 DDD | 263 FHHTYGPRTKWH |
| WaaO               | E. coli R2  | 127 VLYLDADIIQCTTEFLP | 181 YFNSGFLILN | 220 DDD | 263 FHHTYGPRTKWH |

**HexIII transferases**

| WaaJ               | S. enterica  | 127 LLYLDADAVVCKGSSLQEL | 175 YFNNGVVPVN | 213 DDD | 260 LIHHTYGTKWH |
|--------------------|--------------|--------------------------|---------------|---------|------------------|
| WaaI               | E. coli K-12 | 126 LLYLDADAVVCKGSSLQEL | 175 YFNNGVVPVN | 213 DDD | 260 LIHHTYGTKWH |
| WaaR               | E. coli R2  | 126 LLYLDADAVVCKGSSLQEL | 175 YFNNGVVPVN | 213 DDD | 260 LIHHTYGTKWH |

**Related proteins**

| WaaS               | E. coli R2  | 87 LLYLDADIVCNGPDLQEL | 138 YFNGVYTFIN | 174 DDD | 215 IMMYTVQNKFW |
|--------------------|--------------|------------------------|---------------|---------|------------------|
| LgTC               | N. gonorrhoeae | 97 VLYLDADIVCRGKLFL | 150 YFNNGVLLN | 187 DDD | 241 VSYHCGKAPW |
| LgTC               | N. meningitidis | 97 VLYLDADVVRDSLGF | 150 YFNNGVLLN | 187 DDD | 241 VSYHCGKAPW |
| LgtC               | H. influenzae | 107 MYTIDCDAVLDIKSL | 156 YFNNGVLLN | 188 DDD | 241 VSYHCGKAPW |
| GapA               | B. subtilis  | 107 MYTIDCDAVLDIKSL | 156 YFNNGVLLN | 188 DDD | 241 VSYHCGKAPW |
| Wbbm               | K. pneumoniae | 321 VYFGDVDTSVKGDGL | 451 YFQAIIFPN | 486 DDD | 358 MIHYAENKFW |
| LpcA               | R. leguminosarum | 88 LLYLDADVAVLPVL | 143 YFNAGVLFD | 180 DDD | 214 LHIPTGRKPKW |

**FIG. 3. Consensus features of the WaaIJ family of putative α-glycosyltransferases.** Sequences I through IV identify regions of similarity shared by members of a family of glycosyltransferases that are related to the WaaI and WaaJ proteins of S. enterica. Alignment was performed using the ClustalX program. The * indicates positions that have a fully conserved residue. The : indicates conservation of one of the following "weakly conserved" groups: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHRK, NEQHRK, FVLIM, or HFY. The numbers indicate the amino acid position of the first residue of the motif sequence.
and *S. enterica* core OSs both have a side branch α1,2-linked GlcNAc substitution on the terminal Glc residue (Fig. 1A). A Hepp residue occupies the same position in *E. coli* K-12 which would explain the absence of a *waaK* homolog in its core OS biosynthesis gene cluster. *S. enterica* mutations mapping to *waaK* lack GlcNAc in their outer core OS (36), and the *waaK* gene has been identified in this organism (7). The predicted products of the *waaK* homologs from *E. coli* R2 and *S. enterica* share 75.3% identity (83.2% total similarity) (Fig. 1B). The predicted molecular mass of the *E. coli* R2 *waaK* protein is 42.8 kDa based on sequence analysis, and the cloned R2 *waaK* coding region (pWQ901) directs synthesis of a protein with a predicted molecular mass of 43 kDa in Coomassie-stained SDS-polyacrylamide gels (data not shown). The *WaaK* proteins from *E. coli* F632 and *S. enterica* have the EX23E α-glycosyltransferase motif also found in WaaG and WaaB. The motif identified in *E. coli* R2 WaaK is E\(^{8854}\)AFCMVA\(^{906}\), identical to that of *S. enterica* WaaK. Sequence data and structural similarities in the core OSs are consistent with *WaaK* homologs encoding α1,2-linked GlcNAc transferases. Unambiguous assignment was achieved by structural and biochemical analyses of a precisely defined *waaK* mutant in the R2 core OS prototype strain, *E. coli* F632 (see below).

The *E. coli* R2 *waaK* Gene Product Encodes a UDP-N-Acetylglucosamine:(Glucosyl) LPS α1,2-N-Acetylglucosaminyltransferase for Outer Core OS Assembly—Insertional inactivation of the R2 *waaK* coding region in *E. coli* F632 gave strain CWG300 (*waaK::aacC1*). In SDS/Tricine-PAGE, the LPS lipid A-core band of CWG300 migrates slightly faster than that of the wild-type parent (Fig. 4), reflecting a truncated core OS devoid of the terminal GlcNAc moiety. These results suggest that acceptor may well be a limiting factor. However, activities from F632 (2.95 pmol/μg soluble extract protein/h) and CWG300 (pWQ901) (2.96 pmol/μg/h) were over 5-fold higher than the activity observed from CWG300 (0.56 pmol/μg/h). Control experiments indicated that the low level of background activity in the control extract (CWG300) was attributable to the membrane fraction itself and was not dependent on added soluble extract. This may reflect use of UDP-GlcNAc to assemble other cell wall components. Taken together, the structural and biochemical data show that the *waaK* gene from *E. coli* F632 encodes the UDP-N-acetylglucosamine:(glucosyl) LPS α1,2-N-acetylglucosaminyltransferase involved in addition of the terminal side branch in the outer core OS.

The Role of WaaK in Ligation of O-PS—An *S. enterica* mutant with the *waaK953* allele (SL733) does not produce any detectable S-LPS in silver-stained SDS-PAGE (7). The same result is shown in Fig. 6A, lane 2. Introduction of a plasmid containing the complete *waaK* coding region from *S. enterica* was able to complement the *waaK953* phenotype by restoring synthesis of O-PS in this strain (7). As might be expected given the similarities in core OS structures and WaaK homolog sequences, the R2 *waaK* gene carried on pWQ901 could functionally replace the *waaK* gene of *S. enterica* SL733, leading to restoration of S-LPS formation (Fig. 6A, lane 3).

These results suggest that the WaaK added α1,2-linked GlcNAc residue may be required in a functional LPS acceptor for ligated O-PS. However, previous structural analysis of the LPS

![Fig. 4. SDS-PAGE analysis showing the effect of the *waaK::aacC1* mutation on the LPS profile of *E. coli* F632. LPS samples of F632, CWG300, and CWG300(pWQ901) were separated and silver-stained. These strains produce only R-LPS, and only the relevant portion of the gel is shown.](image-url)
from the prototype *S. enterica* waaK953 mutant indicates that the mutant is still able to ligate a trace amount of O-PS to lipid A-core (36). This result can be interpreted in one of two ways: (i) WaaK activity is essential for ligation, but the waaK953 mutation is leaky and retains some enzymatic activity; or (ii) WaaK activity is important for ligation, but its absence only alters the efficiency of the process. These alternatives could be addressed using the *waaK::aacC1* null mutation in *E. coli* CWG300. Due to the fact that *E. coli* F632 produces an R-LPS, a test O-PS was introduced into this strain to study the contribution of core structure to “capping” with O-PS. Plasmid pWQ3 contains all genes necessary for the production of the O-PS (D-galactan I) of *Klebsiella pneumoniae* O1 (20). As shown in Fig. 6, B and C, lane 1, the R2 core of *E. coli* F632 serves as an efficient acceptor for n-galactan I. However, as with *S. enterica*, the GlcNAc-deficient core OS of CWG300 (*waaK::aacC1*) is incapable of acting as acceptor for O-PS (Fig. 6B and C, lane 2). No S-LPS (reflecting ligated O-PS) was detected in SDS-PAGE of LPS samples, either by silver staining or by using the more sensitive Western immunoblotting approach. Introduction of plasmid pWQ901 into CWG300 restores the wild-type ligation-proficient phenotype (Fig. 6B and C, lane 3). From these data, the requirement for the α-1,2-linked Glc pNAc in ligation seems to be essential.

**Characterization of the *E. coli* F632 WaaL Gene Product—** The *waaL* homolog in *E. coli* F632 was initially identified by its occupation of a similar position within the core OS biosynthesis cluster as those in *E. coli* K-12 and *S. enterica*. The predicted R2 WaaL protein is, however, much more similar to the *S. enterica* WaaL protein (81.1% total similarity) than the *E. coli*
K-12 WaaL protein (33.6% total similarity). Hydrophilicity plots of the three WaaL homologs show significant similarity in their predicted structures, and those for *S. enterica* and *E. coli* R2 are virtually identical (Fig. 7). Computer analysis predicts that all three WaaL proteins contain at least eight membrane spanning domains. The distribution and the sizes of the transmembrane segments and surface-exposed loops are similar. The predicted *E. coli* K-12 WaaL protein is slightly larger in size (419 amino acids, 46,874 Da) than WaaL homologs of either *E. coli* R2 (405 amino acids, 46,048 Da) or *S. enterica* (404 amino acids, 46,031 Da).

Inactivation of the *waaL* gene in both *E. coli* K-12 and *S. enterica* results in full-length core OS that is not “capped” by O-PS (7, 37). Similarly, inactivation of the *E. coli* F632 *waaL* gene in strain CWG302 results in the inability of the organism to ligate α-galactan I O-PS to lipid A-core (Fig. 8A, lane 2). Introduction of plasmid pWQ902 into CWG302 restores the ability to ligate α-galactan I to lipid A-core (Fig. 8A, lane 3).

Whereas plasmid pWQ902 readily complements the *waaL* phenotype in *S. enterica* (Fig. 8B, lane 3), it appears not to be able to fully complement the defect in the *E. coli* K-12 *waaL* mutant strain CS2334 (Fig. 8C, lane 3). The amount of α-galactan I that is ligated to lipid A-core in CS2334(pWQ902) is significantly less than that seen in the parent K-12 strain, AB1133, and is only clearly evident when Western immunoblotting is used to detect the S-LPS product (Fig. 8C, compare lanes 1 and 3).

**DISCUSSION**

Variations in outer core OS structures currently determine five different core types in the LPSs of *E. coli* and one in *S. enterica*. The data presented here establish that the *E. coli* R2 core OS biosynthesis gene cluster is a hybrid of those of *E. coli* K-12 and *S. enterica*. The predicted glycosyltransferases (WaaG, -O, and -R) for assembly of the outer core OS backbone are highly similar in *E. coli* K-12 and R2. In contrast, the products of the *waaK* and *waaL* genes of R2, which are involved in the completion of the core OS and ligation of O-PS, are highly conserved with homologs in *S. enterica*.

Relatively little is known about the mechanism of action of glycosyltransferases, and models lean heavily on the more extensive literature for glycosylhydrolases. As more sequences are available it is apparent that there are several families of α- and β-glycosyltransferases. Members of the family identified in prokaryotes contain four conserved regions of primary sequence (Fig. 3) located in regions of common secondary structure. Interestingly, one eukaryotic member of the family (protein T10M13.14 of *Arabidopsis thaliana*) lacks the sequence III motif. It is striking that where substrates for these proteins are known, the WaaIJ family proteins use UDP-...
hexose (Galp or Glcp), and many are involved with the core region of an LPS or lipo-oligosaccharide molecule (Table III). One, WbbM, is involved in O-PS synthesis, and only one (GspA) has been identified through Gram-positive bacteria. It is not possible to assign catalytic and/or binding residues without more extensive biochemical analysis, but the identification of conserved residues in this family provides the foundation on which such strategies will be based.

There are some potential open reading frames in E. coli K-12 whose functions remain obscure (reviewed in Ref. 2). Based primarily on SDS-PAGE data, the waaL gene has been proposed to play a role in an alternate form of LPS which is separate from those molecules which will become an acceptor for O-PS (2). The “waaS” regions in E. coli R2 and K-12 are relatively poorly conserved, and only remnants remain in S. enterica. Analysis of the structure of the predicted R2 WaaS protein indicates that it is an additional member of the WaaJ family of α-glycosyltransferases (Fig. 3). These motifs are absent in the K-12 WaaS protein. Examination of the available structural data for the R2 and K-12 core OSs indicates they have non-stoichiometric substitutions of the KdoII residue with Glc (3). Mutations in waaQ and waaZ affect core OS phosphorylation, but their precise role is not known (3). Structural features of the R2 WaaS protein are consistent with a transferase that uses UDP-Galp as a donor. The absence of a similar modification in S. enterica core OS is consistent with the absence of a complete open reading frame equivalent to waaS. Ultimately, the waaS genes may require unique designations, but this should await experimental determination of their precise roles in core OS assembly. Two additional open reading frames, waaZ and waaY, are conserved in all three core types. Their role in core assembly is presently unknown although the waaZ gene may also play a role in the production of an alternate form of LPS (2). The role of WaaQ is also unknown although it may function as a HepIIF transferase for inner core biosynthesis, based on limited resemblance to the sequences of other heptosyltransferases (3). Mutations in waaP influence inner core phosphorylation, but its precise role is not known (3).

The waaQ and waaP genes (and gene products) are highly
conserved in all three core types. The identification of conserved sequences for these largely uncharacterized genes in *E. coli* R2 does not shed light on the function of their gene products, and since they are not considered to influence outer core OS carbohydrate structure, their roles are not addressed here. However, structural and genetic information is now available to systematically address these additional questions in core OS assembly.

In the core OS of *S. enterica*, addition of the terminal α1,2-linked GlcNac side branch requires WaaK (7, 36, 39). Membranes of *S. enterica* are known to incorporate GlcNac from UDP-GlcNac (40), but the data directly linking WaaK to the glycosyltransferase activity has been circumstantial. Here, we show that the *E. coli* R2 WaaK homolog is the UDP-N-acetylglucosamine:glucosyl LPS α1,2-N-acetylglicosamine transferase for outer core OS assembly. Structural analysis of the *S. enterica* core OS (36) indicate this substitution is stoichiometric. However, in *E. coli* R2 (this study and Ref. 8), this terminal GlcNac substitution is non-stoichiometric. Possible reasons for this minor difference remain unclear. The literature for the corresponding HexIII substitution in *E. coli* K-12 indicates the presence of terminal α-1,6-heptose, referred to as HeplV (38). As predicted from the structures, the K-12 core OS biosynthesis gene cluster does not contain a homolog of waaK. In *E. coli* K-12, the gene which we have renamed waaU (originally this gene was also referred to as *rfgK*) occupies the identical location to *waaK*. It has previously been suggested that WaaU might still be a GlcNac transferase but one involved in transfer of GlcNac to an undefined location in the inner core OS (2, 37). Interestingly, WaaU contains two α-glycosyltransferase motifs, E$^{228}$RQVKYIYQ$^{235}$ and E$^{261}$IVETLPFD$^{269}$, resembling the two closely occurring E[X]-E motifs found in the C-terminal third of WaaC and WaaF proteins of *E. coli* K-12, R2, and *S. enterica*. This is in contrast to the hexosyltransferases such as WaaG, -B, and -K, which contain only a single copy of the motif. Furthermore, BLASTP searches identify regions of local similarity shared by WaaU and a variety of known and predicted heptosyltransferases, including WaaC and WaaF proteins. Thus, whereas the identity of the glycosyltransferase for the terminal α1,6-linked Hepp (HeplV) side branch in the *E. coli* K-12 core is equivocal, waaU is the most likely candidate.

Currently, little is known of the mechanism by which O-PSs are ligated to the lipid A-core molecule. Insights into the ligation reaction could lead to novel therapeutic agents that prevent the attachment of O-PS and lead to a higher degree of complement-mediated killing by the host. The ligase enzyme is envisioned as a glycosyltransferase with a complex (lipid-linked oligosaccharide) substrate requirement. Motifs found in currently known glycosyltransferases are absent in the ligase protein, as might be expected since the ligase substrate is not a nucleotide diphospho sugar. There is little conservation in the primary sequences of the ligases of *E. coli* R2, *S. enterica* and *E. coli* K-12, although their secondary structures appear to be a conserved feature. Ligases from *E. coli* K-12, R2 (see above) and *S. enterica* (data not shown) all interact with and efficiently ligate the reporter O-PS, β-galactan I, to their respective core OS molecules. Furthermore, the ability of the K-12 ligase protein to interact with a variety of different polysaccharide structures is interesting and suggests a relaxed specificity for the ligated structure. To the extent that biosynthetic DNA data are available, all of the polysaccharides currently known to be ligated to lipid A-core by WaaL are assembled on an undecaprenyl pyrophosphoryl lipid intermediate (reviewed in Refs. 1 and 41). The precise details of the trans-cytoplasmic assembly pathways can vary considerably, and the *E. coli* K-12 ligase efficiently ligates O-PS products from the three currently known pathways (1). The undecaprenyl pyrophosphoryl carrier may provide the conserved feature in the ligated substrate for ligase function.

We are interested in the structural requirements for the acceptor in the O-PS ligation reaction. Core OS structure has a profound effect on ligase specificity. Prior to this work, the only known ligases were those from *S. enterica* and *E. coli* K-12. Comparative analysis of these ligases is complicated by core OS structures that differ in both backbone sequence and terminal side branch substituents. Also, a full collection of precise mutations in core OS assembly as well as individually cloned and expressed genes have not been available to address directly the structural requirements. These limitations prompted the analysis reported here.

Previous cross-complementation data indicate that the *waaL* gene from *E. coli* K-12 cannot complement a ligase-defective mutant *S. enterica*, suggesting structural specificity in terms of the core OS acceptor (37). Studies involving a prototype *S. enterica* *waaK* mutant (SL733) indicate that absence of the terminal α1,2-linked GlcNac abrogates attachment of most O-PS; only trace amounts remained (36). As reported here, and in previous work by others (7), there is no evidence of residual S-LPS in the currently available isolate of *S. enterica* SL733. The nature of the *waaK*953 allele is unknown, leading to questions concerning whether WaaK activity is essential for ligation or only important for ligation efficiency. Complementation of the ligation defect by *waaK* genes from the same organism (7) and from *E. coli* R2 (this work) indicates that the defects are confined to *waaK* in *S. enterica* SL733. In a defined *waaK* null mutant of *E. coli* F632 (strain CWG300), the ligation of a reporter O-PS to the R2 core OS is eliminated, and a terminal side group substitution is therefore essential for ligation activity in *E. coli* R2 and probably in *S. enterica*. Consistent with this conclusion, there is one report of the structure of an O-PS (serotype O104) attached to an R2 core OS, and the WaaK-directed GlcNac side branch was present in stoichiometric amounts in the linkage region of the resulting S-LPS (42). The ability of the *E. coli* R2 *waaK* gene product to efficiently complement the *S. enterica* WaaK-mediated ligation defect rules out any role for core OS backbone structure in determination of acceptor specificity in the group of ligases examined here, since these core OSs differ at the HexII position (i.e. Gal in *S. enterica* and Glc in *E. coli* R2).

Interestingly, in previous work from others, some ligation activity was restored when a plasmid containing *waaL* and *waaU* from *E. coli* K-12 was introduced into an *S. enterica* *waaK* mutant (37). Inactivation of the *waaL* coding region of this plasmid eliminated its inability to complement the *waaK* phenotype of *S. enterica*. This suggests that the "complementation" of the *waaK* defect in fact represented replacement of enough *S. enterica* core OS terminus with that of *E. coli* K-12, to allow the K-12 ligase to functionally replace the *S. enterica* chromosomally encoded WaaL. Unfortunately, the structure of the resulting core was not determined. In light of the published lack of complementation of the *S. enterica* *waaL* mutant with the cloned *E. coli* K-12 *waaL* gene (37), the requirement for a precise side group appeared to be absolute. However, using a plasmid carrying only the R2 *waaL* structural gene, we were able to restore low levels of ligation activity in the *E. coli* K-12 *waaL* mutant (CS2334). The differences in the two studies could reflect differences in sensitivity of detection methods or levels of *waaL* expression; previous studies did not use a defined inducible promoter in complementation plasmids. Based on the data presented here, a terminal side group is critical for this group of ligases, although the precise nature of the residue clearly affects efficiency of ligation. The different efficiencies of
the R2 WaAL protein in ligation to the K-12 and R2/S. enterica core OSs presumably reflect steric hinderance resulting from the replacement of the GlcNAc side branch (present in the native R2 core OS) with a Hep residue (in K-12 core OS).

Acknowledgments—We thank K. E. Sanderson and C. Schnaitman for strains; P. Reeves for discussions regarding nomenclature; and W. Wakarchuk for sharing sequence data prior to publication. We also thank A. Clarke for discussions regarding protein analysis and for critically reviewing the manuscript. The excellent technical assistance provided by Karen Amor is gratefully acknowledged.

REFERENCES

1. Whitfield, C., Amor, P. A., and Koplin, R. (1997) Mol. Microbiol. 23, 629–638
2. Schnaitman, C. A., and Klena, J. D. (1993) Microbiol. Rev. 57, 655–682
3. Raetz, C. R. H. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 59–137, American Society for Microbiology, Washington, D.C.
4. Klena, J. D., Pradel, E., and Schnaitman, C. A. (1992) J. Bacteriol. 174, 4746–4752
5. Joiner, K. A. (1988) Annu. Rev. Microbiol. 42, 201–230
6. Makela, P. H., and Stocker, B. A. D. (1984) in Handbook of Endotoxin (Rietschel, E. T., ed) Vol. 1, pp. 133–138, Elsevier Science Publishers, B.V., Amsterdam.
7. MacLachlan, P. R., Kadam, S. K., and Sanderson, K. E. (1991) J. Bacteriol. 173, 7151–7163
8. Hammerling, G., Luderitz, O., Westphal, O., and Makela, P. H. (1971) Eur. J. Biochem. 23, 331–344
9. Schmidt, G., Fromme, I., and Mayer, H. (1970) Eur. J. Biochem. 14, 357–366
10. Miller, J. H. (1992) A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria, p. 439, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
11. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
12. Binotto, J., MacLachlan, P. R., and Sanderson, P. R. (1991) Can. J. Microbiol. 37, 474–477
13. Donnenberg, M. S., and Kaper, J. B. (1991) Infect. Immun. 59, 4310–4317
14. Hamilton, C. M., Aiden, M., Washburn, B. K., Babiuk, P., and Kashmir, S. R. (1989) J. Bacteriol. 171, 4617–4622
15. Amor, P. A., and Whitfield, C. (1997) Mol. Microbiol. 26, 145–161
16. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
17. Hitchcock, P. J., and Brown, T. M. (1983) J. Bacteriol. 154, 269–277
18. Westphal, O., and Jann, K. (1965) Methods Carbohydr. Chem. 5, 83–91
19. Tsai, G. M., and Frasch, C. E. (1982) Anal. Biochem. 119, 115–119
20. Clarke, B. R., Brenner, D., Keenleyside, W. J., Severn, W. B., Richards, J. C., and Whitfield, C. (1995) J. Bacteriol. 177, 5411–5418
21. Dubois, M., Gilles, A. K., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) Anal. Chem. 28, 167–171
22. Suwarkedeer, J. H., Sloneker, J. H., and Jeanes, A. (1967) Anal. Chem. 39, 1602–1604
23. Leontin, K., Lindberg, B., and Lonngren, J. (1978) Carbohydr. Res. 62, 359–362
24. Cisarow, J., and Kerek, F. (1984) Carbohydr. Res. 131, 209–217
25. Osborn, M. J., and Rothfield, L. I. (1966) Methods Enzymol. 8, 456–466
26. Rohr, T. E., and Troy, F. A. (1980) J. Biol. Chem. 255, 2323–2342
27. Muller, E., Hinckley, A., and Rothfield, L. (1972) J. Biol. Chem. 247, 2614–2622
28. Parker, C. T., Kloer, A. W., Schnaitman, C. A., Stein, M. A., Gottesman, S., and Gibson, B. W. (1992) J. Bacteriol. 174, 2325–2338
29. Kadam, S. K., Rehemtulla, A., and Sanderson, K. E. (1985) J. Bacteriol. 161, 277–284
30. Creeger, E. S., and Rothfield, L. I. (1979) J. Biol. Chem. 254, 804–810
31. Geremia, R. A., Petroni, E. A., Ielpi, L., and Henrissat, B. (1996) Biochem. J. 318, 133–138
32. Wollin, B., Creeger, E. S., Rothfield, L. I., Stocker, B. A. D., and Lindberg, A. A. (1983) J. Biol. Chem. 258, 3769–3774
33. Pradel, E., Parker, C. T., and Schnaitman, C. A. (1992) J. Bacteriol. 174, 4736–4745
34. Carstensius, P., Flock, J.-J., and Lindberg, A. (1990) Nucleic Acids Res. 18, 6128
35. Callebaut, I., Labesse, G., Durand, P., Poupon, A., Canard, L., Chemiller, J., Henrissat, B., and Morgan, J. P. (1997) Cell. Mol. Life Sci. 53, 621–645
36. Hellervquist, C. G., and Lindberg, A. A. (1971) Carbohydr. Res. 16, 39–48
37. Klena, J. D., Ashford, R. S., II, and Schnaitman, C. A. (1992) J. Bacteriol. 174, 7297–7307
38. Holst, O., and Brade, H. (1992) in Bacterial Endotoxic Lipopolysaccharides (Morrison, D. C., and Ryan, J. L., eds) Vol. 1, pp. 134–170, CRC Press, Inc., Boca Raton, FL.
39. Lindberg, A. A., and Hellervquist, C. G. (1973) J. Bacteriol. 105, 57–64
40. Osborn, M. J., and D’Ari, L. (1984) Biochem. Biophys. Res. Commun. 16, 568–575
41. Whitfield, C. (1995) Trends Microbiol. 3, 178–185
42. Gamian, A., Romanowska, E., Ulrich, J., and Defaye, J. (1992) Carbohydr. Res. 236, 185–208
43. Reeves, P. R., Hobbs, M., Valmajo, M. A., Skurnik, M., Whitfield, C., Coplin, D., Kido, N., Klena, J., Maskell, D., Raetz, C. R. H., and Rick, P. D. (1996) Trends Microbiol. 4, 495–503
44. Simon, R., Priefer, U., and Puhler, A. (1983) Bio/Technology 1, 784–791
45. Bachmann, B. J. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) Vol. 2, pp. 2460–2488, American Society for Microbiology, Washington, D. C.
46. Beher, M. G., and Schnaitman, C. A. (1981) J. Bacteriol. 147, 972–985
47. Schweizer, H. P. (1993) BioTechniques 15, 831–833
48. Guzman, L.-M., Belin, D., Carsons, M. J., and Beckwith, J. (1995) J. Bacteriol. 177, 4121–4130
49. Clarke, B. R., and Whitfield, C. (1992) J. Bacteriol. 174, 4614–4621