Identification of an ATP-binding Cassette Transporter for Export of the O-antigen across the Inner Membrane in Rhizobium etli Based on the Genetic, Functional, and Structural Analysis of an lps Mutant Deficient in O-antigen*

Inge Lerouge‡, Toon Laeremans‡, Christel Verreth‡, Jos Vanderleyden‡‡, Andrea Tobin**, and Russell W. Carlson***‡‡

From the ‡Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Kasteelpark Areenberg 20, Heverlee B-3001, Belgium and the **Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602

For O-antigen lipopolysaccharide (LPS) synthesis in bacteria, transmembrane migration of undecaprenyl pyrophosphate-bound O-antigen oligosaccharide subunits or polysaccharide occurs before ligation to the core region of the LPS molecule. In this study, we identified by mutagenesis an ATP-binding cassette transporter in Rhizobium etli CE3 that is likely responsible for the translocation of the O-antigen across the inner plasma membrane. Mutant FAJ1200 LPS lacks largely the O-antigen, as shown by SDS-polyacrylamide gel electrophoresis and confirmed by immunoblot analysis. Furthermore, LPS isolated from FAJ1200 is totally devoid of any O-chain glycolyl residues and contains only those glycolyl residues that can be expected for the inner core region. The membrane component and the cytoplasmic ATP-binding component of the ATP-binding cassette transporter are encoded by wzm and wzf, respectively. The Tn5 transposon in mutant FAJ1200 is inserted in the wzm gene. This mutation resulted in an Inf− phenotype in bean plants.

Rhizobiaceae are Gram-negative bacteria that are able to induce the formation of nitrogen-fixing nodules on roots of leguminous plants. For infection and differentiation of nodules, bacterial determinants including surface polysaccharides are required. LPS is the major structural component of a Gram-negative bacterial outer membrane, and evidence for its importance in plant-microbe interactions is appealing. Various Rhizobium mutants with alterations in LPS are defective in the symbiotic association at different stages of infection and nodule development (1).

LPS consists of lipid A, which anchors it to the outer membrane, and a polysaccharide portion that extends into the environment. The polysaccharide portion contains an inner core region, conserved among related strains, and an O-antigen region, whose structure varies in a strain-dependent manner. The O-antigen region consists of the repeating unit and a non-repeating sequence, also referred to as the O-chain attachment region or outer core region (2–5). LPS II (or rough LPS) refers to lipid A and the inner core, whereas LPS I (or smooth LPS) refers to the complete structure. The recent elucidation of the glycosyl sequence of the Rhizobium etli CE3 LPS O-antigen completed the glycosyl sequence of the R. etli CE3 LPS (2–5). The O-antigen polysaccharide was found to be a unique, relatively low molecular weight glycan of a fairly discrete size, with surprisingly little variation in the number of repeating units (degree of polymerization = 5). Each trisaccharide repeating unit consists of glucuronic acid, fucose, and 3-O-methyl-6-deoxytalose (2).

No specific information on the biosynthetic mechanism leading to the assembly of the O-polysaccharide in R. etli CE3 is available. Nevertheless, despite the diversity in structures of O-antigens, the mechanisms involved in their synthesis seem to be conserved in those bacteria that have been studied to date (6). In general, the activated sugar precursors are not transferred directly to a growing LPS molecule. Instead, O-antigens are synthesized separately on a lipid carrier, termed bactoprenyl phosphate. This polymerization step can occur either in the cytoplasm or in the periplasm depending on the assembly pathway. In each case, translocation of the O-antigen across the inner plasma membrane is required. So far, three assembly pathways are known for the polymerization and export of O-antigens. These processes are designated the “Wzy-dependent” pathway, the “ABC transporter-dependent” pathway, and the “synthase-dependent” pathway based on the proteins that are involved in the pathways and the components involved in export across the plasma membrane (7). Once completed, the O-antigen is covalently ligated to a preformed acceptor composed of lipid A and the inner core at the periplasmic face of the plasma membrane. After ligation, the completed LPS molecule is translocated to the cell surface by an unknown mechanism.

The genes involved in saccharide processing, including export, polymerization, and assembly of complex polysaccharides such as LPS, have already been identified in several Enterobacteriaceae. They have all been given names of the form wz* (8).
In *Rhizobium*, however, similar genes have not yet been identified. Nevertheless, at least five genomic regions that seem to have a role in the biosynthesis of LPS in *Rhizobium* were identified (9–16). Most of the information is located in a stretch of the *R. etli* CE3 chromosome, termed the *lpsa* region, in which nine complementation groups have been identified, spanning 17 kilobases of DNA (14).

In this work, we report on the isolation of two genes that are likely involved in the export of rhizobial O-antigenic polysaccharides across the inner membrane. The genetic analysis of a Tn5 mutant (FAJ1200) with a rough colony morphology on agar plates led to the identification of two genes, *wzm* and *wzt*, the deduced amino acid sequences of which show similarities to known ABC-2 transporters or traffic ATPases. Both genes coding for this ABC transporter are located in the previously identified a-region on the chromosome of *R. etli* CE3. Furthermore, the LPS produced by this mutant was structurally analyzed. FAJ1200 LPS is totally devoid of any O-chain glycosyl residue and contains only those glycosyl residues that should be expected for the inner core region. This suggests that the non-repeating sequence or O-chain attachment region is also synthesized as part of the O-chain polysaccharide.

**EXPERIMENTAL PROCEDURES**

**Bacteria and Growth Media**—Escherichia coli strains were maintained on LB agar at 37 °C and grown in LB broth supplemented with the appropriate antibiotics (17). *R. etli* CE3, CE168, mutant FAJ1200, and the trans-conjugant FAJ1206 (mutant FAJ1200 with plasmid pFAJ1248 containing the *R. etli* *wzm* and *wzt* genes) were maintained on Tryptone/yeast medium with added calcium dichloride (18) and supplemented with the appropriate antibiotics. Triparental conjugations were done as previously described (19).

**Nucleic Acid Manipulations and Analysis**—Nucleic acid manipula-
tions were done as previously described (20, 21). Plasmid DNA was prepared, and DNA sequencing was determined with a Tn5 primer, the universal pUC primer, and the reverse pUC primer on an ALF automated sequencer (Amersham Pharmacia Biotech, Uppsala).

PCR Amplification—The 2.4-kb DNA fragment that contains the \textit{wzm} and \textit{wzt} genes was amplified by PCR with platinum \textit{Pfx} DNA polymerase (Life Technologies, Inc.) using two primers: 5\textsuperscript{9}AG-GCGCCGCAGGGAAATACGCCGGGGCG-3\textsuperscript{9} (RHI-120) and 5\textsuperscript{9}TCCCCCGGGCACTGCGACATCGACGACCGCGCG-3\textsuperscript{9} (RHI-141). The 2.4-kb PCR product was ligated to the pFAJ1708 vector (22).

Data Base Searching, Multiple Alignments, and Generation of Phylogenetic Trees—Data base searches were performed using the programs BLASTX and BLASTP (23). The ClustalW algorithm (24) was used for multiple protein alignments, and the alignment layout was prepared by the Genedoc program (25). Phylogenetic trees were generated using the neighbor-joining method (26). Statistical significance values were evaluated with the bootstrapping method (27). Trees were displayed graphically using the software package Treecon for Windows (28).

Plant Assays—\textit{Phaseolus vulgaris} cv. Negro Jamapa seeds were surface-sterilized and germinated as described previously (29). Bean seedlings were planted in jars containing a Jenssen medium agar slant (30). The seedlings were inoculated with 200 \(\mu\)l of an overnight rhizobial culture. The plants were maintained in a growth chamber at 28 °C day and 24 °C night temperatures over a 12-h photoperiod. After 3 weeks, plants were harvested. Uninoculated control plants did not show any nodules or nodule-like structures.

Microscopic Analysis of Nodules—Three-week-old nodules were fixed and embedded in Technovit 7100 matrix (Heraeus Kulzer, Wehrheim, Germany) as described previously (29). Sections of 3 \(\mu\)m were cut on an HM 360 microtome (Microm, Walldorf, Germany) and subsequently stained with toluidine blue. Photographs were taken with a Optiphot-2 microscope using an FX-35DX camera (Nikon, Tokyo).

LPS Purification—LPS was purified by hot phenol/water extraction, followed by size-exclusion chromatography in the presence of deoxycholate (31) using Sephadex G-150 (32–34), unless otherwise stated.

Analysis of the Core Oligosaccharides by High Performance Anion Exchange Chromatography (HPAEC)—The purified lipopolysaccharides were fractionated and analyzed by high performance anion exchange chromatography (HPAEC) using a CarboPac PA100 column (Dionex, Sunnyvale, CA). The eluent was 50 mM NaOH at 1 mL/min. Fractions containing the core oligosaccharides were collected and concentrated.

FIG. 3. Multiple protein sequence alignment of Wzt from \textit{R. etli} CE3 and similar ATP-binding proteins from saccharide transport systems. The alignments were generated by ClustalW (24). Shadings were obtained using the Genedoc program (25). Black indicates 100% identical or conserved (D/N, E/Q, S/T, K/R, F/Y/W, L/I/V/M) residues; dark gray indicates 80% identical or conserved residues; and light gray indicates 60% identical or conserved residues. Gaps introduced for optimal alignment are marked by dashes. Numbers on the right indicate amino acid positions. The origins of sequences are indicated on the left: Ac, \textit{A. actinomycetemcomitans}; Ae, \textit{A. salmonicida}; Hoa, \textit{H. influenzae}; Nei, \textit{N. meningitidis}; Sm, \textit{S. melliloti}; Ec, \textit{E. coli}; Kp, \textit{Klebsiella pneumoniae}; Bj, \textit{B. japonicum}; Ri, \textit{R. leguminosarum} bv. \textit{viciae}; Ps, \textit{P. aeruginosa}; Re, \textit{R. etli}; Sy, \textit{Synechocystis}; Se, \textit{Serratia marcescens}; Bu, \textit{B. pseudomallei}; My, \textit{M. xanthus}. For GenBank\textsuperscript{TM}/EBI accession numbers, see Table I.
ides from CE3 and FAJ1200 were hydrolyzed in 1% acetic acid at 100 °C for 1 h. The oligosaccharides released by mild acid hydrolysis were analyzed by HPAEC as previously described (4).

Glycosyl Composition Analysis—The compositions of the purified lipopolysaccharides were determined by the preparation and gas chromatographic-mass spectrometric analysis of trimethylsilyl methylglycosides as previously described (39).

RESULTS

Isolation of the LPS Mutant FAJ1200—From a large mutant collection, we isolated a Tn5-induced mutant of *R. etli* CE3 (FAJ1200) that had a dry or rough appearance on Tryptone/yeast agar and that also showed a tendency to agglutinate in liquid medium. These properties are usually associated with lipopolysaccharide defects in *Rhizobium*.

Phenotypic Analysis of FAJ1200—*P. vulgaris* cv. Negro Jamapa seedlings were inoculated with wild-type *R. etli* CE3 and the FAJ1200 mutant to determine the nodulation phenotype of the plant. The nodules induced by the mutant were small and white and did not fix nitrogen. Microscopic analysis of 3-week-old nodules was performed. After examination of toluidine blue-stained 3-μm sections, no infected cells could be observed in the nodules induced by the mutant. Vascular bundles were located centrally (Fig. 1B) rather than peripherally as in a normal nodule (Fig. 1A). This symbiotic behavior is

![Fig. 3—continued](http://www.jbc.org/)

![Fig. 4. Phenylogenetic dendrogram showing relative distances between Wzt-like proteins by the neighbor-joining method.](http://www.jbc.org/)
identical to that of the previously reported lps mutant CE168 (Fig. 1C) (14).

Identification of wzt, Which Encodes the Cytoplasmic ATP-binding Component—To identify the gene disrupted by the Tn5 insertion, Southern hybridization was performed on PstI-digested genomic DNA of the mutant with a probe corresponding to the kanamycin resistance gene of Tn5. PstI has a restriction site in the transposon but not the kanamycin resistance gene. A 3.5-kb PstI hybridization signal was detected. The corresponding fragment was cloned into pUC19, and the resulting recombinant plasmid was designated pFAJ1220. The nucleotide sequence of the DNA region flanking the Tn5 transposon was determined with pFAJ1220 as the template DNA and a primer derived from the Tn5 inverted repeat sequence. Sequence analysis was further completed with subclones of the pFAJ1220 insert DNA in pUC19 vector DNA using the universal and reverse pUC primers. The determined sequences revealed similarity to genes encoding the ATP-binding protein of ABC-2 transporters or traffic ATPases. ABC-2 transporters are constituents of the export system for polysaccharides in diverse bacteria. They are composed of two proteins: an inner membrane protein (Wzm) and a cytoplasmic ATP-binding protein (Wzt). Two subunits of each protein probably compose a functional ABC-2 transport system (40).

The identified ORF (ORF2 in Table I) showed similarity to two distinct classes of proteins: those responsible for LPS export and those responsible for capsular export. The first class represents proteins essential for O-antigen transport in Myxococcus xanthus, Pseudomonas aeruginosa, Burkholderia pseudomallei, E. coli O9, Actinobacillus actinomycetemcomitans, and Aeromonas salmonicida. The second class comprises proteins important for the ATP-driven capsular polysaccharide export in E. coli K54, Sinorhizobium meliloti, Haemophilus influenzae, and Neisseria meningitidis. Based on these sequence similarities, the gene corresponding to ORF2 in R. etli CE3 has been named wzt according to the newly implemented bacterial polysaccharide gene nomenclature scheme (8) (Fig. 2).
represented in Fig. 4. Although not all the functions of the listed gene products have been determined, two branches corresponding to the two described classes of Wzt-like proteins can be observed.

Of all the ATP-binding proteins reported, the _R. etli_ CE3 Wzt protein most closely resembles a KpsT-like protein of _Synechocystis_ sp. (29% amino acid identity) and an ABC transporter of _M. xanthus_ (26% amino acid identity). It also shows good similarity to AbcA of _A. salmonicida_, which forms an atypical ABC transporter for O-antigen transport (41). The conserved sequence determinants, the Walker A and Walker B motifs (42) common to all ATP-requiring proteins, are also present in Wzt of _R. etli_ (Fig. 3).

Identification of _wzm_, Which Encodes the Membrane Component—In other bacteria, the _wzm_ gene is located upstream of _wzt_. We knew from previous hybridization experiments (data not shown) that a 7.8-kb EcoRI fragment is located upstream of _wzt_. Therefore, we constructed in pUC19 a size-fractionated library with clones containing _EcoRI_ inserts of ~7.8 kb. Positive clones were selected by Southern hybridization with a DNA probe containing the first 100 base pairs of the _wzt_ gene of _R. etli_ CE3, which overlaps with the 7.8-kb _EcoRI_ fragment. Subcloned fragments from one positive clone were subjected to automated sequence analysis and revealed an ORF (ORF1 in Table I) that showed similarity to Wzm proteins in other bacteria (Fig. 5). The gene corresponding to ORF1 of _R. etli_ CE3 has been named _wzm_. The predicted gene product is similar to the KpsM-like protein of _Synechocystis_ sp. (27% amino acid identity), Wzm of _P. aeruginosa_ (22% identity), and Wzm of _B. pseudomallei_ (22% identity) (43). As for Wzt, we postulate the existence of two classes of Wzm-like proteins based on the results of a phylogenetic analysis (Fig. 6) of the amino acid sequences.

Although the sequence similarities of Wzm proteins are rather low, the structure of Wzm is highly conserved. The hydrophobicity profile (data not shown) indicates that _R. etli_ CE3 Wzm contains six putative membrane-spanning domains, with its N and C termini protruding into the cytoplasm. In addition, we identified a small hydrophobic domain that resides between helices V and VI (Fig. 5) and postulate that it is also situated within the cytoplasmic membrane as reported earlier for KpsM from _E. coli_ K1 (44). This was designated as the SV–SVI linker. It has been assumed that the transmembrane domains of ABC transporters form a pore-like structure required for substrate transport across the cell membrane (45), and it is tempting to speculate that the SV–SVI linker region of Wzm may also function as part of a pore for polymer transport.

To our knowledge, no other _wzm_ or _wzt_ homologs in _Rhizobium_ have been reported. Prieler and Prechel (46) reported the isolation of two chromosomally encoded ORFs in _Rhizobium leguminosarum_ bv. _viciae_ strain VF39 with similarities to proteins involved in the export of polysaccharides, but no sequences were deposited in public data bases.

**ABC Transporter for LPS Assembly in Rhizobium etli**

**FIG. 6.** Phylogenetic dendrogram showing relative distances between Wzm-like proteins by the neighbor-joining method. Numbers represent the bootstrapping score over 100 trials. Only scores above 50 are indicated. The scale at the top of the tree corresponds to 10% divergence between species. See the legend to Fig. 3 for species abbreviations.

**FIG. 7.** 18% deoxycholate-polyacrylamide gel and immunoblot analysis of _R. etli_ FAJ1200 and CE3. Lane 1, LPS from the _R. etli_ FAJ1200 aqueous layer from phenol/water extraction; lane 2, LPS from the _R. etli_ FAJ1200 phenol layer from phenol/water extraction; lane 3, _R. etli_ CE3 LPS. A, silver-stained 18% deoxycholate-polyacrylamide gel; B, immunoblot using mAb JIM26; C, immunoblot using mAb JIM27; D, immunoblot using mAb JIM28; E, immunoblot using mAb JIM32. The table shows the binding of the specific antibodies. +++, strong binding by the antibody; +++, +, medium binding effects; +, weak binding; −, no binding.
FAJ1200 phenol extract is structurally different from parental LPS I in that it failed to bind JIM28. Also, as may be expected, Fig. 7D shows that for both the FAJ1200 phenol and aqueous extracts and for parental CE3 LPS, the LPS II fractions all stained with mAb JIM32, which is specific for the inner core region of \textit{R. leguminosarum} (and \textit{R. etli}) LPS (49). These results indicate that LPS II from FAJ1200, the major LPS produced by this mutant, has the same core structural features that are present in parental CE3 LPS.

To prove that the phenotype of mutant FAJ1200 was due to the mutation in the \textit{wzm} locus and not the result of polar effects on putative downstream genes, a complementation experiment was done. Wild-type \textit{wzm} and \textit{wzt} genes were first amplified by PCR, yielding a PCR fragment of 2.4 kb, and subsequently cloned into the cloning vector pFAJ1708 carrying the promoter (22), resulting in pFAJ1248. pFAJ1248 was mobilized into the FAJ1200 mutant to determine if supplying the genes in \textit{trans} could restore the wild-type phenotype. As shown in Fig. 8, the complemented mutant FAJ1206 carrying pFAJ1248 (containing the PCR-amplified \textit{wzm} and \textit{wzt} genes) was able to synthesize LPS I as the parental strain (lane 3), thus demonstrating that the mutant phenotype observed was due to \textit{wzm} mutation. Nevertheless, it can be noticed that the ratio between LPS I and LPS II in the complemented mutant differs (more LPS II) from the ratio observed in the wild-type strain. Interestingly, a similar phenotype (LPS II \textit{>} LPS I) was observed for two \textit{R. etli} mutants (CE395 and CE394) with \textit{wzm} mutations downstream of the \textit{wzt} gene (50). Since we do not know the detailed genetic structure of the \textit{wzm}/\textit{wzt} downstream region, it is still possible that the mutation in \textit{wzm} has a slight polar effect on genes farther downstream. Nevertheless, a clone containing only \textit{wzm} and \textit{wzt} restores the capacity of the FAJ1200 mutant to produce LPS I and substantiates the finding that \textit{wzm} and \textit{wzt} are indeed responsible for O-antigen export.

**HPAEC Analysis of FAJ1200 LPS**—Fig. 9 shows the core HPAEC profiles obtained from parental CE3 and mutant FAJ1200 purified LPS II. Both the parental and mutant mild acid hydrolysates contain the expected core tri- and tetrasaccharides. This result is consistent with the immunological data using JIM32, which show that FAJ1200 LPS II contains those core structural features found in parental LPS II. In addition, the HPAEC results for FAJ1200 LPS II also indicate the presence of an additional peak not found in parental LPS. This peak is monomeric Kdo, which is also released from FAJ1200 LPS during mild acid hydrolysis. This Kdo residue is due to the external Kdo in the core region that is attached to Gal\textit{\textbeta} of the tetrasaccharide. In parental LPS, the O-chain is attached to that Kdo residue; and therefore, it is not observed in the HPAEC profile. However, in the mutant, that Kdo residue does not have the attached O-chain; and as a result, monomeric Kdo is liberated during mild acid hydrolysis and is observed in the HPAEC profile. Finally, FAJ1200 LPS II is contaminated (peak *) slightly by non-LPS oligosaccharides (see below).

**Composition Analysis**—Table II shows the results of glycosyl composition analysis of FAJ1200 purified LPS II compared with parental CE3 LPS. The results show that FAJ1200 LPS II is totally devoid of any O-chain or outer core glycosyl residues and contains only those glycosyl residues that should be expected for the inner core region. The higher level of mannose and the presence of glucose in LPS II of FAJ1200 are due to a non-LPS contaminant (Fig. 9, peak *) since this glucose/mannose-containing component does not bind to a polymyxin B-Sepharose affinity column on re-purification.

**DISCUSSION**

We have presented evidence that inactivation of an ABC transporter in \textit{R. etli} CE3 drastically affects the structure of LPS in that it prevents addition of the O-antigen polysaccha-
ride to the inner core region. In the ABC transporter-dependent pathway, the glycosyl residues are added one at a time to the nonreducing end of the growing polysaccharide, which is attached to the undecaprenyl phosphate carrier and, once polymerized, is transported to the periplasm via an ABC transporter and then ligated to the lipid A inner core region. As mentioned in the Introduction, two other pathways are known for the polymerization and export of O-antigens (7). The Wzy-dependent system is the classical pathway first described in Salmonella enterica serogroups A, B, D, and E. Recently, Keenleyside and Whitfield (51) reported a third O-antigen biosynthetic pathway called the synphase-dependent pathway for the assembly of the poly-N-acetylmannosamine O-antigen (factor 54) of S. enterica sv. borreze.

The structure of the O-antigen repeating unit is strain-dependent. In R. etli CE3, it is a heteropolymer of a trisaccharide unit consisting of glucuronic acid, fucose, and 3-O-methyl-6-deoxytalose (2). Therefore, the isolation of a wzt-homologous gene responsible for the export of such a heteropolymorphic O-antigen in R. etli CE3 is of interest since most of the bacterial polysaccharide biosynthetic genes encoding ABC-2 transporters are involved in the biosynthesis of homopolysaccharides (6). Other known exceptions are the gene cluster of A. actinomycetemcomitans involved in the biosynthesis of the heteropolymorphic serotype b-specific polysaccharide antigen and the heteropolymeric type II O-polysaccharide gene cluster of B. pseudomallei, which also contains the wzm and wzt genes (43, 52).

Some features of the O-antigen structure of R. etli CE3 further support the operation of an ABC transporter-dependent pathway, as evidenced in this study. The CE3 O-chain polysaccharide is characterized by its very discrete size, i.e. five repeating units, and by the presence of methylated sugars. Other polysaccharides that are synthesized by monomeric addition of glycosyl residues to the nonreducing end and that would utilize the ABC transporter-dependent pathway are reported to contain methylated capping residues, such as those from Klebsiella O5 (53) and E. coli O8 (54).

Wzt shows similarity to NodI from R. leguminosarum bv. viciae, Bradyrhizobium japonicum, S. meliloti, and Rhizobium sp. NGR234. On the basis of both their sequence similarity to traffic ATPases and their organization in an operon together with the nodA, nodB, and nodC genes, the NodI and NodJ proteins have been implicated in the secretion of lipochitooligosaccharide molecules. Evidence for such a role has been obtained by McKay and Djordjevic (55) and Spank et al. (56). However, lipochitooligosaccharides are not transported exclusively via NodIJ because a low level secretion of O-antigen was observed in the nodIJ mutant. An explanation for this could be the operation of a redundant nod factor secretory complex. For example, in S. meliloti, the nolFGHI genes were proposed to constitute such an alternative transporter (57). However, this hypothesis has not yet been confirmed experimentally (58). A second possibility is transporter “cross-talk”: the idea that transporters such as KpsM and KpsT devoted to secretion of capsular polysaccharides could also secrete Nod factors. However, an E. coli kpsM or kpsT mutant carrying the nodABC genes of Azorhizobium caulinodans is able to secrete the produced lipochitooligosaccharide molecules (59). In relation to our study, it could be speculated that such transporter cross-talk results in the inefficient transport of O-antigenic polysaccharide, therefore explaining the trace amounts of LPS I in the phenol phase of the phenol/water extraction from R. etli FAJ1200. It is not known why the small amount of LPS I that is made by FAJ1200 is extracted into the phenol rather than the aqueous phase. However, recently, another LPS I that fails to bind JIM28 but still binds JIM26 and JIM27 also could not be extracted into the aqueous phase. This LPS I was present in a CE3 mutant selected for its inability to bind JIM28 and was not extracted into the aqueous phase (60). Interestingly, also a small amount of O-antigen was detectable by dot immunoblotting from the wzt mutant (formerly called the rfbD mutant) of Yersinia enterocolitica when whole bacteria were applied to the filter (61).

The structural analysis of the mutant LPS shows that the mutation in FAJ1200 gives rise largely to an LPS that does not have the O-chain polysaccharide. Even though a small amount of LPS I was found, it was present only in the phenol layer of the hot phenol/water extract and is structurally different from CE3 LPS I in that it fails to bind mAb JIM28. The mAb JIM28 epitope disappears when the bacteria are grown in the presence of low O₂, at low pH, or in the presence of an anthocyanin isolated from seed extract, mimicking the symbiotic interaction (62). The above results also show that LPS II produced by the mutant is identical to parental LPS II in its PAGE mobility and in its binding to mAb JIM32. The HPAEC analysis revealed that mutant LPS II contains the same core oligosaccharide as that of the parent. The structure of LPS produced by the FAJ1200 mutant and the fact that the mutant is affected in a gene encoding an ABC transporter suggest that the non-replicating unit is also synthesized as part of the O-chain polysaccharide on the undecaprenyl phosphate carrier. Additionally, the anomeric configuration of the distal Kdo residue of the inner core region is α, suggesting that attachment of this residue falls within the domain of the typical core region biosynthetic machinery (2).

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ABC Transporter for LPS Assembly in Rhizobium etli

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Identification of an ATP-binding Cassette Transporter for Export of the O-antigen across the Inner Membrane in *Rhizobium etli* Based on the Genetic, Functional, and Structural Analysis of an *lps* Mutant Deficient in O-antigen

Inge Lerouge, Toon Laeremans, Christel Verreth, Jos Vanderleyden, Caroline Van Soom, Andrea Tobin and Russell W. Carlson

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