Characterization of the Serogroup O11 O-Antigen Locus of *Pseudomonas aeruginosa* PA103

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We previously cloned a genomic DNA fragment from the serogroup O11 *Pseudomonas aeruginosa* strain PA103 that contained all genes necessary for O-antigen synthesis and directed the expression of serogroup O11 antigen on recombinant *Escherichia coli* and *Salmonella*. To elucidate the pathway of serogroup O11 antigen synthesis, the nucleotide sequence of the biosynthetic genes was determined. Eleven open reading frames likely to be involved in serogroup O11 O-antigen biosynthesis were identified and are designated in order as *wzz*_{PaO11} (*wzz* from *P. aeruginosa* serogroup O11), *wzx*_{PaO11}, *whjA*, *wzy*_{PaO11}, *whjB* to *whjF*, *wbpL*_{O11} and *wbpM*_{O11} (*wbpL* and *wbpM* from serogroup O11). Consistent with previous descriptions of O-antigen biosynthetic gene loci, the entire region with the exception of *wbpM*_{O11} has a markedly reduced G+C content relative to the chromosomal average. *Wzy*_{PaO11} shows no significant similarity at the protein or DNA sequence level to any database sequence and is very hydrophobic, with 10 to 12 putative transmembrane domains, both typical characteristics of O-antigen polymerases. A nonpolar chromosomal insertion mutation in *wzy*_{PaO11} in *P. aeruginosa* PA103 confirmed the identity of this gene. There is striking similarity between *WhjBCDE* and *Cap(5/8)EFGL*, involved in type 5 and type 8 capsule biosynthesis in *Staphylococcus aureus*. There is nearly total identity between *wbpM*_{O11} and *wbpM*_{OS}, previously shown by others to be present in all 20 *P. aeruginosa* serogroups. Using similarity searches, we have assigned functions to the proteins encoded by the PA103 O-antigen locus and present the potential steps in the pathway for the biosynthesis of *P. aeruginosa* serogroup O11 O antigen.

Pseudomonas aeruginosa is an opportunistic pathogen that expresses a number of virulence factors. One of these factors, lipopolysaccharide (LPS), is the immunodominant antigen responsible for serogroup specificity. The LPS is composed of three parts: lipid A, embedded in the membrane; a core oligosaccharide; and the variable O antigen (also called B-band LPS), composed of repeating oligosaccharide units that extend out from the cell surface. There are 20 different serogroup antigens of *P. aeruginosa* that differ in their monosaccharide composition and/or linkages between sugar residues. *P. aeruginosa* has an additional rhamnan-containing polysaccharide component, termed common antigen (also referred to as A-band LPS) (21).

P. aeruginosa serogroup O11 has one of the simplest O-antigen structures (22): [3)- α -L-FucNAc-(1-3)- β -D-FucNAc-(1-2)- β -D-Glc-(1-)]. Serogroup O11 strains are common in the environment and in hospital outbreaks and have recently been shown to exhibit multidrug resistance (13, 48). The serogroup O11 strain PA103 has been used for identification and characterization of numerous virulence factors and in animal and tissue culture models of infection (20, 29, 40, 50). We previously reported the cloning of the O-antigen locus of *P. aeruginosa* PA103 and the surface expression of *P. aeruginosa* serogroup O11 antigen on *Escherichia coli* (16). In a mouse model,

oral vaccination with an attenuated *Salmonella typhimurium* strain expressing *P. aeruginosa* serogroup O11 has been shown to protect against oral challenge with a *P. aeruginosa* serogroup O11 strain (33).

The biosynthetic pathway for the production of LPS is complex. Genes for synthesis of the lipid A, LPS core, and O side chain components are often found in separate regions of the chromosome. The genes encoding the enzymes for the synthesis of O antigen (previously referred to as *rfb* genes) are usually clustered and may form an operon. It is interesting that the G+C content of these regions is often significantly lower than that of the rest of the chromosome, suggesting that genes for O-antigen synthesis may have been acquired by horizontal gene transfer from organisms with a lower G+C content. O-antigen synthesis involves a number of genes encoding products for the production of nucleotide sugar precursors, for the transfer of the sugars into the O-antigen polymer, and for the linkage of the O-antigen unit onto the LPS core to form the complete LPS (36).

One of the final steps in the synthesis of full-length O antigen is the polymerization of individual O-antigen subunits by O-antigen polymerase, encoded by the *wzy* (previously referred to as *rfc*) gene. Inactivation of the *wzy* gene results in a strain wherein the LPS core is capped with a single O-antigen unit. This phenotype is referred to as semirough or core-plus-one. In general, all *wzy* genes have the following characteristics: a lower G+C content and skewed codon bias compared to the rest of the chromosome (although it may be similar to other linked genes involved in O-antigen synthesis) and a gene product that is predicted to be highly hydrophobic, with 11 to 13 transmembrane domains. It is interesting that even with these

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristics	Reference or source
<i>P. aeruginosa</i>		
PA103	IATS serogroup O11	25
PAO1	IATS serogroup O5	23
AK1401	O-antigen polymerase mutant of PAO1, LPS core-plus-one O-antigen subunit	3
AK44	LPS-specific-bacteriophage-resistant mutant of PAO1, LPS complete core	23
<i>E. coli</i>		
DH5 α	<i>supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA</i>	Gibco BRL
SM10	<i>thi-1 thr leu tonA lacY supE recA</i> RP4-2-Tc::Mu, Km ^r	45
Plasmids		
pEX100T	Mobilizable, counterselectable gene replacement vector; Ap ^r /Cb ^r , sucrose susceptible	43
pUCGM	Contains <i>aacC1</i> ; Ap ^r , Gm ^r	42
pUCP18/19	High-copy-number <i>E. coli</i> / <i>P. aeruginosa</i> shuttle vectors; Ap ^r /Cb ^r	41
pMO012437	Contains DNA from the 39-min region of PAO1 in the broad-host-range cosmid pLA2917	35
pMO011618	Contains DNA from the 40-min region of PAO1 in the broad-host-range cosmid pLA2917	35
pLAFR1	Broad-host-range cosmid; Tc ^r	14
pLPS2	pLAFR1 containing an approximately 26.2-kb DNA fragment from <i>P. aeruginosa</i> PA103 encompassing the serogroup O11 O-antigen gene cluster	16
pLPS224	pLAFR1 harboring an approximately 2.4-kb <i>Asp718I-HindIII</i> fragment from pLPS2 containing the <i>wbpL</i> _{O11} gene	12
pGEM226	pGEM3 (Promega Corp.) harboring an approximately 4.4-kb <i>NsiI-EcoRI</i> fragment derived from pLPS2; contains the 3' end of <i>wbpL</i> _{O11} , <i>tRNA-asnV</i> , <i>tyrB</i> , and <i>wbpM</i> _{O11}	This study
pCF100	pGEM226 <i>EcoRI-NcoI</i> deletion derivative; contains <i>tRNA-asnV</i> and <i>tyrB</i>	This study
pCF101	pEX100T harboring an approximately 1.6-kb <i>EcoRI-HindIII</i> fragment from pCF100; contains <i>tyrB</i>	This study
pCF102	pCF101 containing the <i>aacC1</i> gene from pUCGM, inserted into the unique <i>XhoI</i> site in <i>tyrB</i>	This study
pCD100	pUCP19 harboring an approximately 3.5-kb <i>Asp718I-XhoI</i> fragment derived from pLPS2 encompassing <i>wzy</i> _{PaO11}	This study
pCD101	pCD100 with the <i>aacC1</i> gene from pUCGM inserted into the unique <i>BssHII</i> site in <i>wzy</i> _{PaO11}	This study
pCD102	pEX100T harboring an approximately 4.7-kb <i>PvuII</i> fragment derived from pCD101, encompassing <i>wzy</i> _{PaO11} :: <i>aacC1</i>	This study
pCD103	pUCP18 harboring a 2.2-kb <i>EcoRI-Asp718I</i> fragment derived from pCD100 containing <i>wzy</i> _{PaO11}	This study

similarities, there is no obvious homology between *wzy* genes from different organisms or from different families of serogroups from the same organism. In *P. aeruginosa*, the *wzy* gene of strain PAO1 (serogroup O5) was identified by complementation of the serum-sensitive (i.e., killed in the presence of 10% normal human serum) phenotype of the core-plus-one mutant of PAO1, AK1401 (7). We and others noted the low G+C content of this gene, the hydrophobic nature of the inferred amino acid sequence, the potential transmembrane regions, and the distribution of this gene only in strains that share a structurally related O-repeat backbone (7, 10). In this report, we present the nucleotide sequence analysis of the O-antigen gene locus from *P. aeruginosa* PA103. We recognized 11 open reading frames (ORFs) and confirmed that one of them corresponds to the O-antigen polymerase. On the basis of similarities to other gene products involved in polysaccharide biosynthesis, we propose a pathway for the synthesis of *P. aeruginosa* serogroup O11 O antigen.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The bacterial strains and plasmids used in this study are listed in Table 1. Luria (L) broth or agar was used for routine growth of bacteria. Cetrimide agar (Difco Laboratories, Detroit, Mich.) was used as a selective medium for *P. aeruginosa*. Minimal medium (18) with glucose (20 mM), supplemented with tyrosine, phenylalanine, and tryptophan (1 mM each) as required, was used for determination of auxotrophy in *P. aeruginosa*. The media contained ampicillin (50 to 100 μ g/ml), carbenicillin (250 μ g/ml), gentamicin (15 μ g/ml for *E. coli*; 250 μ g/ml for *P. aeruginosa*), tetracycline (10 μ g/ml for *E. coli*; 70 to 100 μ g/ml for *P. aeruginosa*), sucrose (5% wt/vol), isopropyl- β -D-thiogalactopyranoside (IPTG; 40 μ g/ml), and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal; 40 μ g/ml) as required.

DNA manipulations. Plasmid DNA was isolated with Wizard Plus Minipreps (Promega Corp., Madison, Wis.) or plasmid kits (Qiagen Inc., Chatsworth, Calif.). Genomic DNA was isolated by the method of Woo et al. (51) or with the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.). Restriction endonucleases and modification enzymes (Boehringer Mannheim Corp., Indianapolis, Ind.) were used as specified by the manufacturer.

Plasmids were introduced into *E. coli* as previously described (8). The plasmids were transformed into *P. aeruginosa* by the electroporation protocol of Smith and Iglewski (47). Agarose gel electrophoresis was performed as previously described (8). Following agarose gel electrophoresis, DNA fragments were isolated with the QIAquick or QIAEX II gel extraction kit (Qiagen) as specified by the manufacturer. Pulsed-field gel electrophoresis (PFGE) was performed on a RAGE apparatus (Stratagene, La Jolla, Calif.) as previously described (15).

For Southern blots, restriction endonuclease-digested genomic DNA was separated on 0.7% agarose gels and transferred to a GeneScreen (NEN Life Science Products, Boston, Mass.)-charged nylon membrane as previously described (17) or with the Vacugene XL vacuum blotting system (Pharmacia Biotech, Piscataway, N.J.) as directed by the manufacturer. DNA probe labelling, hybridization, and detection were carried out with the ECL direct nucleic acid labelling and detection system (Amersham Life Science, Cleveland, Ohio) as specified in the protocol provided with the kit. For Southern blotting after PFGE, plasmid DNA was labelled with ³²P by nick translation and used as a probe.

Oligonucleotide primers used in PCR and nucleotide sequence determinations were purchased from the Great American Gene Co. or Ransom Hill (Ramona, Calif.). PCR was performed with the EasyStart PCR Mix-in-a-Tube (Molecular Bio-Products, Inc., San Diego, Calif.) and a Perkin-Elmer GeneAmp PCR System 2400 thermocycler as specified by the manufacturer. Nucleotide sequence determinations of both strands of plasmid pLPS2 insert DNA were carried out by the Molecular Medicine Unit at Beth Israel Hospital (Boston, Mass.) and the Biomolecular Research Facility (University of Virginia). The sequence upstream of *wzz*_{PaO11} (*wzz* from *P. aeruginosa* serogroup O11), including *himD*, was obtained from a PCR fragment amplified from *P. aeruginosa* PA103 chromosomal DNA. Nucleotide sequence fragments were assembled and analyzed with the Sequencher (Gene Codes Corp., Ann Arbor, Mich.), Gene Inspector (Textco Corp., West Lebanon, N.H.), and GCG version 9 (Genetics Computer Group, Inc., Madison, Wis.) computer programs. GenBank database searches (National

FIG. 1. Genetic organization of the *P. aeruginosa* serogroup O11 strain PA103 O-antigen biosynthetic gene cluster. The numbers in brackets indicate the G+C content of each gene in moles percent. The site of insertion of the *Gm^r* determinant (*aacCI*) in *wzyPaO11* is shown. The arrows represent the limits of each ORF and the predicted direction of transcription. A, *Asp* 718I; Bs, *Bss*HII; E, *Eco*RI.

Center for Biotechnology Information, Bethesda, Md.) were conducted by the FASTA search protocol of Pearson and Lipman (32).

In vitro mutagenesis and gene replacement. A nonpolar mutation of *wzyPaO11* was constructed in vitro by insertion of a gentamicin resistance gene (*aacCI*), recovered as an 879-bp *Sma*I fragment from plasmid pUCGM, into the unique *Bss*HII site (rendered blunt) in *wzyPaO11* on plasmid pCD100. *wzyPaO11::aacCI* was then recovered from the resultant plasmid (pCD101) as an approximate 4.7-kb *Pvu*II fragment and ligated to *Sma*I-digested pEX100T. This construct (pCD102) was introduced into the mobilizing *E. coli* strain SM10, and SM10(pCD102) was conjugated with *P. aeruginosa* PA103 by pelleting approximately equal numbers of donor (grown overnight at 37°C) and recipient (grown overnight without shaking at 42°C) strains in microcentrifuge tubes and spotting them onto the center of an L agar plate. After incubation for 12 to 18 h at 37°C, the cells were resuspended in 1 ml of L broth and plated on gentamicin-containing cetrimide plates. Colonies arising after 48 h were purified on the same medium and then swabbed onto L agar plates containing both gentamicin and sucrose. Since pEX100T contains the *sacB* gene, which renders gram-negative bacteria sensitive to sucrose, colonies arising on sucrose- and gentamicin-containing plates carried *wzyPaO11::aacCI* on the chromosome and had lost the vector-associated *sacB* gene. These colonies were tested for sensitivity to carbenicillin to confirm the loss of vector DNA. Gene replacement was further confirmed by PCR amplification of chromosomal DNA with primers flanking the *aacCI* insertion site in *wzyPaO11*. The PCR product from *P. aeruginosa* PA103 *wzyPaO11::aacCI*, estimated by agarose gel electrophoresis, was larger than that generated from wild-type chromosomal DNA by an amount consistent with the size of the *aacCI* gene. Southern blot analysis of *Asp*718I-digested chromosomal DNA from the mutant and wild-type strains with *wzyPaO11* as the probe showed a similar increase in hybridizing-band size for the mutant strain.

For disruption of *tyrB* on the chromosome, an approximately 1.6-kb *Eco*RI-*Hind*III fragment from pCF100, containing the *tyrB* gene, was excised, rendered blunt, and ligated to pEX100T. The resulting construct (pCF101) was linearized at the unique *Xho*I site within *tyrB*, and rendered blunt; into it was ligated the *Sma*I fragment containing the gentamicin resistance gene from pUCGM, to yield pCF102. Plasmid pCF102 was introduced into the mobilizing *E. coli* strain SM10, and conjugation and gene replacement into *P. aeruginosa* strains PA103 and PAO1 were carried out as described above.

Isolation of LPS. *P. aeruginosa* LPS was isolated as previously described (8) or by the proteinase K digestion method of Hitchcock and Brown (19), followed by hot-phenol extraction with slight modifications. Briefly, cells grown overnight on L agar plates with appropriate antibiotics were resuspended in phosphate-buffered saline to an optical density at 600 nm of 0.6. Cells from 4.5 ml of the suspension were collected by centrifugation, resuspended in 200 µl of lysis buffer (2% sodium dodecyl sulfate [SDS], 4% β-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue in 1 M Tris [pH 6.8]), and boiled for 15 min. The lysed cells were treated with RNase and DNase for 30 min at 37°C and digested for 3 h at 59°C with 120 µg of proteinase K. The digested lysates were then extracted with an equal volume of 90% phenol for 15 min at 65°C with periodic vortexing. After centrifugation in a microcentrifuge at maximum speed for 10 min, both the aqueous and phenol layers were transferred to new tubes and extracted with 1 ml of diethyl ether. The aqueous layers from these extractions were removed, pooled, and again extracted with hot phenol. The aqueous layer (blue) was again extracted with 1 ml of ether and centrifuged, and the LPS-containing aqueous layer was diluted 1:3 with fresh lysis buffer and used for Tricine-SDS-polyacrylamide gel electrophoresis (PAGE) analysis.

Tricine-SDS-PAGE and Western immunoblotting. LPS samples were separated by Tricine-SDS-PAGE, as previously described (8), on 17% (37.8:1 acrylamide-bis-acrylamide) running gels. LPS was visualized by silver staining with a silver-staining kit (Bio-Rad Laboratories, Hercules, Calif.) as specified by the manufacturer. After separation by Tricine-SDS-PAGE, LPS was transferred to a NitroBind nitrocellulose membrane (Micron Separations Inc., Westboro, Mass.) by using the Trans-Blot semidry electrophoretic transfer cell (Bio-Rad Laboratories) as specified by the manufacturer.

Western immunoblot analyses were performed as previously described (8).

The antibodies used were monoclonal antibody (MAb) specific to *P. aeruginosa* O11 or O5 LPS (Rougier Bio-Tech Ltd., Montreal, Quebec, Canada), common antigen-specific MAb N1F10 (purchased from J. S. Lam, University of Guelph, Guelph, Ontario, Canada), or polyclonal antisera to *P. aeruginosa* O11 or O5 LPS (34). The blots were developed at room temperature with anti-mouse immunoglobulin M-alkaline phosphatase (Boehringer Mannheim Corp., Indianapolis, Ind.) for the MAb and anti-rabbit immunoglobulin G-alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) for the polyclonal antisera. Sigma Fast 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium tablets (Sigma Chemical Co.) were used as the alkaline phosphatase substrate as specified by the manufacturer.

Nucleotide sequence accession number. The sequence from *himD* to *tyrB* appears in the EMBL/GenBank/DBJ nucleotide sequence data libraries under accession no. AF147795.

RESULTS AND DISCUSSION

Mapping of the O-antigen gene locus. PFGE was used to map the 26.2-kb fragment of PA103 DNA that was previously shown to encode all the genes necessary for *P. aeruginosa* serogroup O11 O-antigen synthesis to the *P. aeruginosa* chromosome. PFGE maps of strain PA103 and two other strains have been compared with the physical map of strain PAO1 (44). *Spe*I-digested fragments from *P. aeruginosa* PA103 and PAO1 were separated by PFGE and probed with pLPS2. The probe hybridized to the largest *Spe*I fragment (585 kb) in strain PA103 and to a fragment of approximately 260 kb in strain PAO1. The 260-kb *Spe*I fragment of PAO1 has been mapped to 39 to 40 min on the physical linkage map of the chromosome (35). To determine whether pLPS2 hybridized to the same region of the chromosome in strain PA103 and PAO1, defined cosmid clones (pMO012437 from the 39-min region and pMO011618 from the 40-min region of PAO1 [35]) were used to probe PFGE-separated fragments of both strains. The cosmid clones hybridized to the appropriate *Spe*I fragment in PAO1 (400 and 260 kb, respectively) and to the largest *Spe*I fragment in PA103, thus indicating that the O-antigen locus of PA103 is in the 39- to 40-min region of the *P. aeruginosa* chromosome, the same region to which the PAO1 O-antigen locus has been previously localized (7, 24).

Sequence analysis of the *P. aeruginosa* serogroup O11 O-antigen genes and predicted proteins. Figure 1 shows the *P. aeruginosa* serogroup O11 O-antigen biosynthetic gene cluster. The O-antigen locus consists of 11 potential ORFs all transcribed in the same direction and residing between the housekeeping genes *himD* and *tyrB*, essentially the same location as the previously described serogroup O5 O-antigen locus of *P. aeruginosa* PAO1 (4). However, all serogroup O11 ORFs appear to be involved in O-antigen biosynthesis, unlike the serogroup O5 locus, where the *hisH* and *hisF* genes and the insertion element *IS1209* were found within the O-antigen gene cluster (4). O-antigen gene clusters typically have a G+C content more than 10% lower than the chromosomal average of

the organism (36). Consistent with this observation, all of the serogroup O11 O-antigen genes, with the exception of *wbpM* from serogroup O11 (*wbpM*_{O11}), have G+C contents (Fig. 1) at least 10% lower than the *P. aeruginosa* chromosomal average of 65 to 67 mol% (7, 30, 49).

The best results of comparisons of each deduced serogroup O11 O-antigen biosynthetic protein against protein databases by using the FASTA search strategy (32) are summarized in Table 2. Of the 11 identified ORFs, 10 are predicted to encode proteins with significant similarities to bacterial proteins previously found to be involved in synthesis of surface polysaccharides, including LPS O antigens of gram-negative bacteria and capsular polysaccharides of gram-positive bacteria.

O-antigen transport and assembly genes: *wzz*, *wzx*, and *wzy*. The deduced amino acid sequence of *wzz*_{PaO11} has about 41% identity to *Wzz*_{PaO5} of *P. aeruginosa* PAO1 and 21 and 23% identity to *WzzE* of *Salmonella typhimurium* and *E. coli*, respectively, which suggests that this gene encodes the *P. aeruginosa* serogroup O11 O-antigen chain length determinant (Table 2). At the nucleotide level, *wzz*_{PaO11} and *wzz*_{PaO5} have 55% identity. *Wzz*_{PaO11} has an overall hydrophilic character but does exhibit strong predicted transmembrane regions at both the N and C termini, characteristic of *Wzz* proteins in general (5) and suggesting that they are localized to the inner membrane. Using *TnpA*, Morona et al. (28) have shown that the cloned *Shigella flexneri* *Wzz* is an integral membrane protein whose central hydrophilic portion is located in the periplasmic space, where it is proposed to interact with the O-antigen polymerase (*Wzy*) and/or ligase (*WaaL*) proteins. Burrows et al. (5) have disrupted the *wzz* genes in *P. aeruginosa* serogroup O5 and O16 strains, resulting in an altered distribution of O-antigen chain lengths. They also showed that *wzz*_{PaO5} could regulate the chain length of the heterologous O antigen of *Shigella dysenteriae*, extending previous observations that *Wzz* proteins are not O-antigen specific (1, 2, 5).

The deduced amino acid sequence of *wzx*_{PaO11} is 26% identical to *E. coli* RfbX (*Wzx*) and 22% identical to the *Yersinia enterocolitica* *Wzx* proteins (Table 2), suggesting that *wzx*_{PaO11} encodes the *P. aeruginosa* O11 O-antigen transporter (flippase). As is characteristic of flippases in general, *Wzx*_{PaO11} is very hydrophobic (grand average of hydropathicity [GRAVY] = 1.084) and exhibits 11 or 12 potential transmembrane domains. *Wzx* proteins are involved in the transport of completed O-antigen subunits across the inner membrane; disruption of *wzx* in *E. coli* led to the accumulation of undecaprenol phosphate (Und-P)-linked O subunits in the cytoplasm (26). Of particular note, *Wzx*_{PaO11} shows 22% identity to Cap8K (Table 2), which is proposed to be a transporter of type 8 capsule polysaccharide in *Staphylococcus aureus*. This sequence similarity may reflect the presence of a common fucosamine disaccharide residue in the *S. aureus* capsule and the *P. aeruginosa* serogroup O11 O antigen.

A gene within the sequence of the *P. aeruginosa* PAO1 O-antigen gene locus, *wbpF*, was recently reanalyzed and found to be the serogroup O5 O-antigen transporter; it was therefore redesignated *wzx* (6). *WbpF* did not appear in a FASTA search comparing *Wzx*_{PaO11} to the database; similarly, this newly recognized protein showed no significant similarity to *Wzx*_{PaO11}.

The *wzy*_{PaO11} gene had no significant homology at either the DNA or protein sequence level to any sequence deposited in the databases that were searched. The predicted protein is very hydrophobic (GRAVY = 1.067) and has 10 or 12 predicted transmembrane domains. The distribution of the transmembrane domains is similar to that in the previously described *Wzy*_{PaO5}, the serogroup O5 O-antigen polymerase (7).

Since the putative *Wzy*_{PaO11} protein shows no significant homology to database sequences, nonpolar insertional inactivation of *wzy*_{PaO11} was undertaken to verify its function. *P. aeruginosa* PA103 *wzy*_{PaO11}::*aacC1* cells produce a truncated LPS consisting of the core substituted with one O-antigen repeating unit, which is indicative of an inability to polymerize O-antigen repeating units (Fig. 2A to C, lanes 2). The presence of one repeating unit indicates that O-antigen subunit synthesis is unaffected by disruption of *wzy*_{PaO11}. Restoration of full-length LPS production by plasmid-borne *wzy*_{PaO11} (lanes 3) confirmed both the role of *Wzy* in polymerizing O-antigen subunits and the nonpolarity of the gentamicin resistance determinant inserted into the *wzy* gene in *P. aeruginosa* PA103 *wzy*_{PaO11}::*aacC1*. Thus, *wzy*_{PaO11} encodes the serogroup O11 antigen polymerase. Consistent with observations of *wzy*_{PaO5} mutants (10), interruption of *wzy*_{PaO11} does not affect production of the D-rhamnan common antigen (Fig. 2D), indicating that *Wzy*_{PaO11} plays no apparent role in synthesis of this component of the LPS.

It is characteristic of O-antigen polymerases that they exhibit little or no homology to one another (27), except in cases where different serogroup O antigens form a chemically and structurally related family (11). This is not surprising since they are involved in recognizing and polymerizing different polysaccharide subunits that define structurally distinct O antigens. The observed lack of sequence homology between the *Wzy* proteins of *P. aeruginosa* PA103 (serogroup O11) and PAO1 (serogroup O5), whose O antigens are structurally dissimilar, supports the notion that the differences in sequence reflect substrate specificity. What is less clear is why the proteins differ so completely in primary structure when their function and probably their structure are conserved. Whether this difference reflects the involvement of most of the primary amino acid sequence in direct interaction with different cognate substrates and/or specific transport and assembly proteins that may have coevolved or is an indication that the *wzy* genes are each acquired from evolutionarily distant sources (and therefore even the structurally and functionally conserved regions could differ substantially in primary structure) remains to be seen. The latter case is in keeping with the proposed idea that serogroup-specific O antigen genes in general may often be acquired, at least in part, from distantly related organisms (see below).

In view of the apparent unrelatedness of *Wzy*_{PaO11} and *Wzy*_{PaO5}, these proteins would not be expected to be functionally interchangeable. Indeed, plasmid pCD103, containing *wzy*_{PaO11} gene, complements *P. aeruginosa* PA103 *wzy*_{PaO11}::*aacC1* (Fig. 2) but does not complement the *P. aeruginosa* PAO1 serogroup O5 O-antigen polymerase mutant, AK1401, as determined by Western immunoblotting. When plasmid pCD103 was reisolated from strain AK1401(pCD103), it could still restore full-length LPS production in PA103 *wzy*_{PaO11}::*aacC1*, confirming the integrity of plasmid pCD103 in strain AK1401 (data not shown). This indicates that the *Wzy* proteins probably need to maintain overall structural similarities but must use their primary amino acid sequence to recognize and polymerize their cognate O-antigen subunits.

O-antigen biosynthetic genes, *wbjA* to *wbjF*, *wbpL*_{O11}, and *wbpM*_{O11}. The deduced amino acid sequence of the *wbjA* gene shows significant similarity to various glycosyltransferases (Table 2). The protein is generally hydrophilic (GRAVY = -0.228), but there are one or two potential transmembrane domains.

The deduced amino acid sequences from *wbjB*, *wbjC*, *wbjD*, and *wbjE* exhibit close identity to amino acid sequences corresponding to proteins involved in the synthesis of *Staphylo-*

TABLE 2. *P. aeruginosa* serogroup O11 strain PA103 O antigen biosynthetic proteins: properties and similarities to database sequences^a

Protein	Size (aa ^b /kDa)	Similar proteins	% identity (expectation value)	Putative function	Database accession no. ^c
Wzz _{PaO11}	345/38.5	Wzz _{PaO5} (<i>Pseudomonas aeruginosa</i>)	41 (3.8×10^{-53})	O-antigen chain length determinant	U50397
		WzzE (<i>Salmonella typhimurium</i>)	21 (0.18)	O-antigen chain length determinant	O33789 (SP)
		WzzE (<i>Escherichia coli</i>)	23 (0.45)	O-antigen chain length determinant	P25905 (SP)
		CpsC (partial) (<i>Streptococcus pneumoniae</i>)	31 (0.6)	Capsule polysaccharide chain length determinant	AF030373
		Wzb8 (<i>Escherichia coli</i>)	20 (0.66)	O-antigen chain length determinant	O33953 (SP)
Wzx _{PaO11}	411/44.3	RfbX (<i>Escherichia coli</i>)	26 (7.8×10^{-26})	O-antigen transporter	P37746 (SP)
		Wzx (<i>Yersinia enterocolitica</i>)	22 (4.1×10^{-18})	O-antigen transporter	S51260 (PIR)
		mth347 (<i>Methanobacterium thermoautotrophicum</i>)	26 (6.5×10^{-18})	O-antigen transporter	AE000819
		RfbX (<i>Shigella dysenteriae</i>)	23 (4.4×10^{-15})	O-antigen transporter	Q03583 (SP)
		mth367 (<i>Methanobacterium thermoautotrophicum</i>)	23 (3.6×10^{-11})	O-antigen transporter	AE000822
		EpsK (<i>Lactococcus lactis</i> subsp. <i>cremoris</i>)	20 (9.4×10^{-8})	Similar to O-antigen transporter	U93364
		Cap8K (<i>Staphylococcus aureus</i>)	22 (6.4×10^{-6})	Capsule synthesis	U73374
WbjA	334/37.8	PhcG024 (<i>Pyrococcus horikoshii</i>)	25 (2.8×10^{-9})	Hypothetical protein	AB009478
		PmHAS (<i>Pasteurella multocida</i>)	27 (3.4×10^{-7})	Hyaluronan synthase (glycosyltransferase)	AF036004
		AmsB (<i>Erwinia amylovora</i>)	29 (2.3×10^{-5})	Amylovoran biosynthesis (glycosyltransferase)	Q46632 (SP)
		PssI (<i>Rhizobium leguminosarum</i>)	24 (2.4×10^{-5})	Glycosyltransferase	AF028810
		PssH (<i>Rhizobium leguminosarum</i>)	23 (6.4×10^{-5})	Glycosyltransferase	AF028810
Wzy _{PaO11}	411/46.0				
WbjB	344/38.6	Cap8E (<i>Staphylococcus aureus</i>)	68 (2.2×10^{-92})	Capsule biosynthesis	U73374
		Cap5E (<i>Staphylococcus aureus</i>)	67 (5.5×10^{-92})	Capsule biosynthesis	U81973
		Protein D homolog (<i>Methanococcus jannaschii</i>)	40 (1.5×10^{-44})	Homolog of capsular biosynthetic protein D	D64432 (PIR)
		FlaA1 (<i>Helicobacter pylori</i>)	39 (2.4×10^{-43})	Flagellin synthesis	AE000595
		FlaA1 (<i>Caulobacter crescentus</i>)	39 (3.2×10^{-43})	Flagellin synthesis	U27301
		YpqP (partial) (<i>Bacillus subtilis</i>)	41 (2.2×10^{-21})	Similar to capsule biosynthetic protein	P54183 (SP)
		YodU (<i>Bacillus subtilis</i>)	50 (3.8×10^{-21})	UDP-glucose epimerase	AF015775
WbjC	372/41.4	Hypothetical protein (<i>Acholeplasma laidlawii</i>)	47 (4.6×10^{-72})	Probable nucleotide binding protein	S33518 (PIR)
		Cap8F (<i>Staphylococcus aureus</i>)	42 (2.0×10^{-62})	Capsule biosynthesis	U73374
		Cap5F (<i>Staphylococcus aureus</i>)	42 (4.5×10^{-62})	Capsule biosynthesis	U81973
		PhaM009 (<i>Pyrococcus horikoshii</i>)	23 (2.2×10^{-5})	Vi polysaccharide biosynthesis	AB009524
		Nse (<i>Aquifex aeolicus</i>)	26 (0.0076)	Nucleotide sugar epimerase	AE000735
WbjD	376/42.8	Cap5G (<i>Staphylococcus aureus</i>)	54 (2.4×10^{-81})	Capsule biosynthesis	U81973
		Cap8G (<i>Staphylococcus aureus</i>)	54 (3.7×10^{-81})	Capsule biosynthesis	U73374
		BplD homolog (<i>Methanococcus jannaschii</i>)	31 (3.8×10^{-38})	LPS biosynthesis	G64487 (PIR)
		Mth837 (<i>Methanobacterium thermoautotrophicum</i>)	33 (2.2×10^{-33})	UDP-N-acetylglucosamine-2-epimerase	AE000860
		Ph1617 (<i>Pyrococcus horikoshii</i>)	34 (1.7×10^{-31})	UDP-N-acetylglucosamine-2-epimerase	AP000006
		WbpI (<i>Pseudomonas aeruginosa</i>)	30 (3.7×10^{-30})	LPS biosynthesis-epimerase	U50396
WbjE	400/44.6	Hypothetical protein (<i>Synechocystis</i> sp.)	25 (3.8×10^{-19})	Hypothetical protein	D90901
		Cap5L (<i>Staphylococcus aureus</i>)	25 (1.3×10^{-18})	Capsule biosynthesis	U81973
		Cap8L (<i>Staphylococcus aureus</i>)	25 (1.5×10^{-18})	Capsule biosynthesis	U73374
		BplE (<i>Bordetella pertussis</i>)	23 (4.1×10^{-8})	Glycosyltransferase involved in LPS biosynthesis	S70676 (PIR)
		WbpJ (<i>Pseudomonas aeruginosa</i>)	21 (5.7×10^{-8})	LPS biosynthesis-transferase	U50396

Continued on following page.

TABLE 2—Continued

Protein	Size (aa ^b /kDa)	Similar proteins	% identity (expectation value)	Putative function	Database accession no. ^c
WbjF	314/34.5	WbpK (<i>Pseudomonas aeruginosa</i>)	52 (1.2×10^{-64})	LPS biosynthesis-dehydratase	U50396
		GalE (<i>Vibrio cholerae</i>)	40 (2.9×10^{-43})	UDP-glucose-4-epimerase	Q56623 (SP)
		ORF35x7 (<i>Vibrio cholerae</i>)	43 (5.4×10^{-43})	LPS biosynthesis	Y07788
		WbiG (<i>Burkholderia pseudomallei</i>)	39 (4.9×10^{-35})	Epimerase/dehydratase	AF064070
		HpnA (<i>Zymomonas mobilis</i>)	28 (5.8×10^{-13})	Biosynthesis of hapanoid sugar side chains	AJ001401
		Cap8N (<i>Staphylococcus aureus</i>)	28 (5.7×10^{-11})	Capsule biosynthesis	U73374
WbpL _{O11}	341/37.2	Cap5N (<i>Staphylococcus aureus</i>)	33 (6.7×10^{-11})	Capsule biosynthesis	U81937
		WbpL _{O5} (<i>Pseudomonas aeruginosa</i>)	65 (5.8×10^{-144})	LPS biosynthesis-glycosyltransferase	U50396
		WbcO (<i>Yersinia enterocolitica</i>)	53 (8.4×10^{-73})	Glycosyltransferase (LPS biosynthesis)	S51265 (PIR)
		Llm (<i>Staphylococcus aureus</i>)	27 (7.7×10^{-20})	Involved in methicillin resistance/autolysis	A55856 (PIR)
		TagO (<i>Bacillus subtilis</i>)	25 (2.2×10^{-19})	Und-P N-acetylglucosaminyltransferase (teichoic acid biosynthesis)	AJ004803
		WbiH (<i>Burkholderia pseudomallei</i>)	35 (5.1×10^{-18})	Und-P N-acetylglucosaminyltransferase	AF064070
WbpM _{O11}	665/74.5	WbpM _{O5} (<i>Pseudomonas aeruginosa</i>)	96 (2.7×10^{-259})	LPS biosynthesis-epimerase	U50396
		Orf10 (<i>Vibrio cholerae</i>)	50 (1×10^{-58})	Epimerase/dehydratase	U47057
		WbcP (<i>Yersinia enterocolitica</i>)	49 (1.6×10^{-57})	Galactose modification (LPS biosynthesis)	S51266 (PIR)
		Orf3 (<i>Neisseria meningitidis</i>)	46 (1.2×10^{-53})	Documentation withdrawn	AF014804
		RfbU (<i>Vibrio cholerae</i>)	47 (2.6×10^{-52})	Mannosyltransferase	Y07788
		LpsB2 (<i>Rhizobium etli</i>)	49 (7.8×10^{-42})	dTDP-glucose-4,6-dehydratase	U56723
		WblL (<i>Bordetella pertussis</i>)	46 (1.8×10^{-36})	LPS biosynthesis	S70683
		WbiI (<i>Burkholderia pseudomallei</i>)	40 (6×10^{-29})	Putative epimerase/dehydratase	AF064070
		CapD (<i>Staphylococcus aureus</i>)	40 (6.6×10^{-29})	Capsule biosynthesis	P39853
		Cap5D (<i>Staphylococcus aureus</i>)	39 (7×10^{-28})	Capsule biosynthesis	U81973
		Cap8D (<i>Staphylococcus aureus</i>)	38 (1.3×10^{-27})	Capsule biosynthesis	U73374
		Protein D homolog (<i>Methanococcus jannaschii</i>) ^d	42 (9.7×10^{-21})	Homolog of capsular biosynthetic protein D	D64432 (PIR)
		YpqP (partial) (<i>Bacillus subtilis</i>) ^d	43 (7.9×10^{-12})	Similar to capsule biosynthetic protein	P54183 (SP)
		FlaA1 (<i>Caulobacter crescentus</i>) ^d	32 (3.9×10^{-11})	Flagellin synthesis	U27301
		WbjB (<i>Pseudomonas aeruginosa</i>) ^d	31 (1.5×10^{-9})		AF147795
		Cap8E (<i>Staphylococcus aureus</i>) ^d	32 (2.6×10^{-8})	Capsule biosynthesis	(this study) U73374

^a Nonredundant database searched 8 August 1998.^b aa, amino acids.^c SP, Swiss-Prot; PIR, Protein Identification Resource.^d Aligns over the C-terminal half of WbpM.

coccus aureus capsular polysaccharides (Table 2). The *S. aureus* type 5 and type 8 capsule biosynthetic loci are composed of 16 ORFs denoted *cap5/8A* to *cap5/8P* (transcribed in that order) (39) (see Fig. 3). Two regions, composed of *cap5/8A* to *cap5/8G* and *capL* to *capP*, are essentially identical between the two capsule loci, with nucleotide sequence identities ranging from 97.4 to 99.7%. Flanked by these two common regions, Cap5H to Cap5K contain no significant identity to their Cap8H to Cap8K counterparts and are considered to be the capsule type-specific regions of each of these strains (39).

WbjB is 68 and 67% identical to the Cap8E and Cap5E proteins, respectively. WbjC is 47% identical to a putative nucleotide binding protein described in *Acholeplasma laidlawii*; however, it is also clearly related to Cap8F and Cap5F (42% identity for each). WbjD is related to Cap5G and Cap8G (54% identity for each). WbjE has 25% identity to three proteins, Cap5L, Cap8L, and a hypothetical protein from *Synechocystis* sp. (Table 2).

The relatedness of WbjB to WbjE to components involved in

S. aureus capsule synthesis is also evident at the nucleotide level (Fig. 3), with *wbjB* being approximately 65% identical to *cap8E*, *wbjC* being approximately 55% identical to *cap8F*, and *wbjD* being approximately 57% identical to *cap5/8G*. While *wbjE* does not have significant overall identity to *cap5/8L* to *cap5/8P* does appear on a FASTA nucleotide database search with 64% identity over a 100-nucleotide overlap (expectation = 0.04).

The deduced amino acid sequence of *wbjF* shows the strongest similarity to WbpK from *P. aeruginosa* PAO1 (52% identity) and UDP-glucose-4-epimerase (GalE) from *Vibrio cholerae* (40% identity) (Table 2). WbjF also exhibits less, but still significant, relatedness to Cap5/8N from *S. aureus*. At the nucleotide level, *wbjF* exhibits 57% identity to *wbpK*.

WbjF and WbpM_{O11} (described below), are similar to Cap5/8N and Cap5/8D (and Cap5/8E in the C-terminal portion), respectively, which are common to both *S. aureus* capsule types. These similarities (and those of WbjB to WbjE, described above) suggest that the structures of serogroup O11 O

FIG. 2. Effect of *wzy* on LPS biosynthesis. (A) Silver-stained Tricine-SDS-PAGE gel. (B) Western immunoblot obtained with serogroup O11-specific polyclonal serum. (C) Western immunoblot obtained with serogroup O11-specific MAb. (D) Western immunoblot obtained with the common antigen-specific MAb, N1F10. Lanes: 1, PA103; 2, PA103 *wzy*_{PaO11}::*aacCI*; 3, PA103 *wzy*_{PaO11}::*aacCI*(pCD103). The positions of the core and core-plus-one O-antigen repeating units (arrows) are shown.

antigen and the *S. aureus* type 5 and type 8 capsular polysaccharides should have a common constituent. In fact, all three structures share α -L-FucNAc-(1-3)- β -D-FucNAc as a major component of their overall structures (22, 39).

The deduced amino acid sequence of WbpL_{O11} shows high identity to WbpL_{O5} from *P. aeruginosa* PAO1 (65%) and WbcO from *Y. enterocolitica* (53%) (Table 2). There is also significant identity with the Und-P *N*-acetylglucosaminyltransferase TagO from *Bacillus subtilis* (25%) and WbiH from *Burkholderia pseudomallei*. At the nucleotide level, *wbpL*_{O11} shows significant identity to both *wbpL*_{O5} (61%) and *wbcO* (60%).

Both WbpL_{O5} and WbcO are thought to initiate O-antigen subunit synthesis by adding FucNAc to the lipid carrier Und-P, after which the remaining sugars of the O antigen subunit are processively added (4, 46). Like the other proposed initiating glycosyltransferases, WbpL_{O11} is highly hydrophobic, having a GRAVY of 0.844 and possessing 9 to 11 predicted transmembrane domains. The likely integration of WbpL_{O11} in the inner membrane suggests a role in anchoring O-antigen subunit synthesis to the membrane and possible association with the O-antigen transporter that would translocate the completed O-antigen units across the inner membrane to be polymerized.

The similarity between WbpL_{O11} and WbpL_{O5} suggested

that these two proteins may be functionally interchangeable. Supporting this, plasmid pLPS224 (12), containing *wbpL*_{O11}, was able to complement the serogroup O5 LPS defect in the *P. aeruginosa* PAO1 mutant strain AK44 (data not shown), an LPS-deficient mutant (23) previously used to identify the *wbpL*_{O5} gene from strain PAO1 (9). Moreover, previous work from our laboratory showed that pLPS224 also complements unknown mutations in certain *P. aeruginosa* O-antigen-deficient strains (isolated from the lungs of patients with cystic fibrosis) to restore the expression of serogroup O6 and O9 LPS (12). Consistent with the observed sequence similarity between WbpL_{O11} and WbpL_{O5} and the functional interchangeability of the *wbpL* gene, an internal probe derived from *wbpL*_{O11} hybridized with genomic DNA from all 20 *P. aeruginosa* serogroup strains under low-stringency conditions (data not shown).

While the nature of the mutation in AK44 is not known, it affects only O-antigen expression while leaving common antigen expression intact (9). In contrast, insertional mutagenesis of *wbpL*_{O5} to create a null mutation abolished the expression of both antigens, leading the authors to suggest that WbpL_{O5} is involved in initiation of synthesis of both common and O-antigen repeating units in serogroup O5 strain PAO1 (38).

FIG. 3. Comparison of the serogroup O11 O-antigen synthesis genes of *P. aeruginosa* PA103 (top) and type 5/8 capsular polysaccharide synthesis genes of *S. aureus* (bottom) (GenBank accession no. U73374 and U81973). The identity at both the DNA and protein levels is indicated. Where there are slight differences between type 5 and 8 homologues, the identity with the best expectation value is used (Table 2). The *capH* to *capK* genes are capsule type-specific genes that are not homologous and differ slightly in total size. The asterisk indicates identity *cap5L* over a 100-bp overlap. The shaded areas represent homologies as follows: WbpM is approximately 39% identical to Cap5D and 31% identical to WbiB; Cap5E is 37% identical to Cap5D.

In the serogroup O5 sequence reported, an insertion element, IS1209, was found between *wbpL* and *wbpM*, and it was suggested that this insertion element represents the junction between "serogroup-specific" and "nonspecific" regions of O-antigen gene clusters in *P. aeruginosa* (4). However, no insertion elements were found in the PA103 serogroup O11 O-antigen locus.

The *wbpM*_{O11} gene sequence and its deduced amino acid sequence are virtually identical to those of *wbpM*_{O5} described in *P. aeruginosa* PAO1 (Table 2). Southern blot analysis with a *wbpM*_{O11} internal fragment as probe has confirmed that this gene is common to all 20 *P. aeruginosa* serogroup strains, a homology also noted by Burrows et al. (4). WbpM_{O11} shows extensive identity to the ORF10 product (epimerase/dehydratase) of *V. cholerae* (50%) and WbcP of *Y. enterocolitica* (49%), involved in the synthesis of serotype O:3 LPS outer core. As is the case for many of the serogroup O11 O-antigen biosynthetic proteins (described above), WbpM_{O11} also shows significant similarity to non-serotype-specific proteins involved in *S. aureus* capsule synthesis, particularly CapD (Table 2). The presence of FucNAc in the structures of *S. aureus* type 5 and 8 capsules, *Y. enterocolitica* outer core, and many of the *P. aeruginosa* O antigens suggests that CapD, WbcP, and WbpM_{O11} may all function in the synthesis of this residue. Indeed, Skurnik et al. (46) speculate that WbcP is involved in the biosynthesis of GalNAc or FucNAc. Burrows et al. (4) also suggest that the WbpM proteins of *P. aeruginosa* are involved in making either GalNAc or FucNAc, found in nearly all of the 20 serogroup LPS structures. For serogroup O5 LPS, they propose that WbpM converts UDP-GlcNAc to UDP-GalNAc, which is subsequently processed to UDP-FucNAc by WbpK and WbpB (4). Sau et al. (39) suggest that CapD is an epimerase or dehydratase involved in synthesis of D-FucNAc and that it works in conjunction with other non-serotype-specific Cap proteins in the synthesis of the fucosamine disaccharide common to both type 5 and type 8 capsule structures. WbpM_{O11} may function similarly in conjunction with the above-described WbjB to WbjE proteins (showing extensive homology to Cap proteins [see above]) to synthesize the same disaccharide in the O11 O-antigen subunit.

Consistent with observations reported for WbcP (46), the proteins showing homology to WbpM_{O11} fall into two distinct groups (Table 2). The first contains proteins with a size similar to WbpM_{O11} (665 amino acids) that exhibit strong predicted transmembrane helices within approximately the first 200 amino acids. The second group of proteins are approximately half the size of WbpM_{O11} or less and show significant identity to the C-terminal half of WbpM_{O11} (Table 2). Most of these proteins are involved in sugar processing, with the exception of *Caulobacter crescentus* FlaA1, which is required for the synthesis of flagellin. The presence in WbpM and its homologs of two distinct domains suggests that the WbpM family of proteins may have arisen as fusions, which now localize biosynthesis to the membrane. The distinct potential membrane-spanning regions in the N termini of these proteins imply that localization to the membrane is important for proper function. Proteins involved in sugar conversions in most LPS biosynthetic systems described are usually soluble cytoplasmic proteins (46), suggesting that the action of the WbpM family of proteins is more complex than simple sugar conversion. The consensus that these proteins are probably involved in synthesis of FucNAc, postulated to be the initiating sugar for synthesis of many *P. aeruginosa* serogroup LPS subunits, prompts speculation that WbpM and its homologs are membrane associated to synthesize the initiation sugar and/or transfer it directly to the corresponding membrane-bound initiating glycosyltransferase

(perhaps WbpL) for linking to the lipid carrier Und-P. The involvement of WbpM in the synthesis of O antigen was confirmed by Burrows et al.: a mutation of *wbpM*_{O5} in PAO1 resulted in loss of serogroup O5 O antigen (4).

The nucleotide sequence conservation of WbpM among the 20 *P. aeruginosa* serogroup strains is intriguing. Conservation implies a similar function for this protein in each strain despite the differences in the O-antigen subunits being made in each serogroup. This conserved function may reflect the presence of FucNAc and/or GalNAc in the majority of the O-antigen subunit structures (22), consistent with the proposed involvement of WbpM in the synthesis of these sugars (4). WbpM could also be involved in synthesis of more highly conserved structures such as the LPS core or possibly even non-LPS molecules. Whether mutations in *wbpM* have additional effects on other structures is currently under investigation.

Nucleotide sequence downstream of the O-antigen locus: *asnV*, *tyrB*, and *uvrB* genes. The near total identity observed between the nucleotide sequences of *wbpM*_{O11} and *wbpM*_{O5} continues downstream of this gene. In the PAO1 sequence analysis (4), a gene designated *wbpN* was identified between *wbpM* and *uvrB*. The deduced amino acid sequence of WbpN showed 19.2% identity to homocitrate synthase from *Rhodobacter sphaeroides*, and it was proposed that this protein may be involved in LPS biosynthesis (4). In the PA103 sequence there is no ORF corresponding to *wbpN*, since there is a stop codon in the middle of this region. Moreover, in both PA103 and PAO1, an overlapping ORF whose deduced amino acid sequence shows over 50% identity to *E. coli* (GenBank accession no. P04693) and *S. typhimurium* (GenBank accession no. Z68874) TyrB was identified on the opposite strand. This similarity extends to other aminotransferases including AspC of *E. coli* (47% identity; GenBank accession no. P00509) and *Haemophilus influenzae* (47% identity; GenBank accession no. P44425) and PhhC of *P. aeruginosa* (45% identity; GenBank accession no. P43336). The location of the putative *tyrB* gene corresponds to that of a partial aminotransferase sequence found upstream of the *P. aeruginosa* *uvrB* gene by Rivera et al. (37) (GenBank accession no. X93486). Along with *tyrB*, we noted a tRNA gene showing approximately 95% identity to *E. coli* *asnV* (GenBank accession no. X52792) and located between *wbpM* and *tyrB*. We also found this tRNA gene in the sequence reported for this region in PAO1.

To test whether *tyrB* plays a role in LPS production, *tyrB* mutations in PA103 and PAO1 were constructed. Consistent with the data of Patel et al. (31), these *tyrB* mutants showed a leaky auxotrophic phenotype and required either tyrosine or phenylalanine for growth but did not grow when supplemented with tryptophan. The *tyrB* mutation did not have any obvious effect on the expression of O antigen or common antigen in either PA103 or PAO1 (Fig. 4). The presence of housekeeping genes adjacent to *wbpM* suggests that *wbpM* is probably the terminal gene in both O antigen gene clusters.

Serogroup O11 O-antigen biosynthetic genes were probably horizontally acquired. The identity between the *P. aeruginosa* serogroup O11 O antigen genes *wbjB* to *wbjD* and *capE* to *capG* at both the DNA and protein sequence levels and the similarity of their order and orientation provide strong evidence of the transfer of these genes from *S. aureus* or another as yet uncharacterized low-G+C-containing organism (Fig. 3). Although the similarities are less dramatic, *wbjE* and *wbjF* also appear to have similar foreign origins (Fig. 3). While the method of acquisition of these genes is not known, a section of the *wbjB* nucleotide sequence (nucleotides 230 to 740) exhibits significant identity to the *B. subtilis* bacteriophage SPB attachment (*attB*) site (GenBank accession no. M81761) (58%, ex-

FIG. 4. Effect of *tyrB* on LPS biosynthesis. (A) Silver-stained Tricine-SDS-PAGE gel. (B) Western immunoblot obtained with serogroup O5 (lanes 1 and 2)- or serogroup O11 (lanes 3 and 4)-specific MAb. (C) Western immunoblot obtained with the common antigen-specific MAb, N1F10. Lanes: 1, PAO1; 2, PAO1 *tyrB::aacC1*; 3, PA103; 4, PA103 *tyrB::aacC1*.

pectation value = 1.6×10^{-22}). Moreover, the *B. subtilis* YpqP partial protein (with similarities to capsular polysaccharide proteins) showing similarity to WbjB (Table 2) is reported as partial due to interruption of *ypqP* by insertion of the SPB prophage. A second potential *attB* site also occurs in *wbpM*_{O11} (52% identity over nucleotides 1140 to 1630). The presence of these sites suggests a bacteriophage-mediated event in the transfer of polysaccharide genes and supports a gram-positive origin for these genes. In addition, the homology between WbjB and the C-terminal half of WbpM_{O11} (Table 2) extends to the nucleotide level (approximately 53% identity over a 548-nucleotide overlap). The possibility that these homologous end regions were duplicated during the bacteriophage-mediated insertion of the intervening DNA suggests that at least a portion of *wbpM*, found in all 20 *P. aeruginosa* serogroup strains, may also have outside origins.

Proposed steps for the synthesis of serogroup O11 O antigen. On the basis of the structure of the *P. aeruginosa* serogroup O11 O-antigen unit and the similarity of potential gene products to other gene products involved in polysaccharide biosynthesis, we propose the following steps for the synthesis of this serogroup O antigen. The FucNAc-FucNAc portion of the serogroup O11 O-antigen subunit structure, similar to that found in *S. aureus* type 5 and 8 capsules, is synthesized by the combined actions of WbjB to WbjF. WbjA adds a glucose residue to the FucNAc disaccharide to complete the O subunit. WbpM_{O11} and WbpL_{O11} synthesize the initiating sugar (probably FucNAc) and transfer it to Und-P at the membrane. Like other Wzy-dependent O antigens, the subunits are then transported across the membrane and polymerized by Wzx_{PaO11} and Wzy_{PaO11}, respectively, and are then attached to the LPS core with the O-antigen chain length being controlled by Wzz_{PaO11}.

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REFERENCES

- Bastin, D. A., L. K. Romana, and P. R. Reeves. 1991. Molecular cloning and expression in *Escherichia coli* K-12 of the *rfb* gene cluster determining the O antigen of an *E. coli* O111 strain. *Mol. Microbiol.* **5**:2223–2231.
- Batchelor, R. A., P. Alifano, E. Biffali, S. I. Hull, and R. A. Hull. 1992. Nucleotide sequences of the genes regulating O-polysaccharide antigen chain length (*rol*) from *Escherichia coli* and *Salmonella typhimurium*: protein homology and functional complementation. *J. Bacteriol.* **174**:5228–5236.
- Berry, D., and A. M. Kropinski. 1986. Effect of lipopolysaccharide mutations and temperature on plasmid transformation efficiency in *Pseudomonas aeruginosa*. *Can. J. Microbiol.* **32**:436–438.
- Burrows, L. L., D. F. Charter, and J. S. Lam. 1996. Molecular characterization of the *Pseudomonas aeruginosa* serotype O5 (PAO1) B-band lipopolysaccharide gene cluster. *Mol. Microbiol.* **22**:481–495.
- Burrows, L. L., D. Chow, and J. S. Lam. 1997. *Pseudomonas aeruginosa* B-band O-antigen chain length is modulated by Wzz (Rol). *J. Bacteriol.* **179**:1482–1489.
- Burrows, L. L., and J. S. Lam. 1999. Effect of *wzx* (*rfbX*) mutation on A-band and B-band lipopolysaccharide biosynthesis in *Pseudomonas aeruginosa* O5. *J. Bacteriol.* **181**:973–980.
- Coyne, M. J., Jr., and J. B. Goldberg. 1995. Cloning and characterization of the gene (*rfc*) encoding O-antigen polymerase of *Pseudomonas aeruginosa* PAO1. *Gene* **167**:81–86.
- Coyne, M. J., Jr., K. S. Russell, C. L. Coyle, and J. B. Goldberg. 1994. The *Pseudomonas aeruginosa* *algC* gene encodes phosphoglucomutase, required for the synthesis of a complete lipopolysaccharide core. *J. Bacteriol.* **176**:3500–3507.
- Dasgupta, T., and J. S. Lam. 1995. Identification of *rfbA*, involved in B-band lipopolysaccharide biosynthesis in *Pseudomonas aeruginosa* serotype O5. *Infect. Immun.* **63**:1674–1680.
- de Kievit, T. R., T. Dasgupta, H. Schweizer, and J. S. Lam. 1995. Molecular cloning and characterization of the *rfc* gene of *Pseudomonas aeruginosa* (serotype O5). *Mol. Microbiol.* **16**:565–574.
- de Kievit, T. R., T. Staples, and J. S. Lam. 1997. *Pseudomonas aeruginosa* *rfe* genes of serotypes O2 and O5 could complement O-polymerase-deficient semi-rough mutants of either serotype. *FEMS Microbiol. Letts.* **147**:251–257.
- Evans, D. J., G. B. Pier, M. J. Coyne, Jr., and J. B. Goldberg. 1994. The *rfb* locus from *Pseudomonas aeruginosa* strain PA103 promotes the expression of O antigen by both LPS-rough and LPS-smooth isolates from cystic fibrosis patients. *Mol. Microbiol.* **13**:427–434.
- Farmer, J. J., III, R. A. Weinstein, C. H. Zierdt, and C. D. Brokopp. 1982. Hospital outbreaks caused by *Pseudomonas aeruginosa*: importance of serogroup O11. *J. Clin. Microbiol.* **13**:427–434.
- Friedman, A. M., S. R. Long, S. E. Brown, W. J. Buikema, and F. M. Ausubel. 1982. Construction of a broad host range cosmid cloning vector and its use in the genetic analysis of *Rhizobium* mutants. *Gene* **18**:289–296.
- Goldberg, J. B., K. Hatano, and G. B. Pier. 1993. Synthesis of lipopolysaccharide O side chains by *Pseudomonas aeruginosa* PAO1 requires the enzyme phosphomannomutase. *J. Bacteriol.* **175**:1605–1611.
- Goldberg, J. B., K. Hatano, G. Small-Meluleni, and G. B. Pier. 1992. Cloning and surface expression of *Pseudomonas aeruginosa* O antigen in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **89**:10716–10720.
- Goldberg, J. B., and D. E. Ohman. 1984. Cloning and expression in *Pseudomonas aeruginosa* of a gene involved in the production of alginate. *J. Bacteriol.* **158**:1115–1121.
- Goldberg, J. B., and D. E. Ohman. 1987. Construction and characterization of *Pseudomonas aeruginosa* *algB* mutants: role of *algB* in high-level production of alginate. *J. Bacteriol.* **169**:1593–1602.
- Hitchcock, P. J., and T. M. Brown. 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. *J. Bacteriol.* **154**:269–277.
- Kang, P. J., A. R. Hauser, G. Apodaca, S. M. J. Fleiszig, J. Wiener-Kronish, K. Mostov, and J. N. Engel. 1997. Identification of *Pseudomonas aeruginosa* genes required for epithelial cell injury. *Mol. Microbiol.* **24**:1249–1262.
- Knirel, Y. A., and N. K. Kochetkov. 1994. The structure of lipopolysaccharides of gram-negative bacteria. III. The structures of O-antigens: a review. *Biochemistry (Moscow)*. **59**:1325–1383.
- Knirel, Y. A., E. V. Vinogradov, N. A. Kocharova, N. A. Paramonov, N. K. Kochetkov, B. A. Dmitriev, E. S. Stanislavsky, and B. Lanyi. 1988. The structure of O-specific polysaccharides and serological classification of *Pseudomonas aeruginosa*. *Acta Microbiol. Hung.* **35**:3–24.
- Kropinski, A. M., L. C. Chan, and F. H. Milazzo. 1979. The extraction and analysis of lipopolysaccharides from *Pseudomonas aeruginosa* strain PAO, and three rough mutants. *Can. J. Microbiol.* **25**:390–398.
- Lightfoot, J., and J. S. Lam. 1993. Chromosomal mapping, expression and synthesis of lipopolysaccharide in *Pseudomonas aeruginosa*: a role for guanosine diphospho (GDP)-D-mannose. *Mol. Microbiol.* **8**:771–782.
- Liu, P. V. 1973. Exotoxins of *Pseudomonas aeruginosa*. I. Factors that influence the production of exotoxin A. *J. Infect. Dis.* **128**:506–513.
- Liu, W. S., R. A. Cole, and P. R. Reeves. 1996. An O-antigen processing function for Wzx (RfbX): a promising candidate for O-antigen flippase. *J. Bacteriol.* **178**:2102–2107.

27. Morona, R., M. Mavris, A. Fallarino, and P. A. Manning. 1994. Characterization of the *rfc* region of *Shigella flexneri*. J. Bacteriol. **176**:733–747.
28. Morona, R., L. Van Den Bosch, and P. A. Manning. 1995. Molecular, genetic, and topological characterization of O-antigen chain length regulation in *Shigella flexneri*. J. Bacteriol. **177**:1059–1068.
29. Ohman, D. E., J. C. Sadoff, and B. H. Iglewski. 1980. Toxin A-deficient mutants of *Pseudomonas aeruginosa* PA103: isolation and characterization. Infect. Immun. **28**:899–908.
30. Palleroni, N. J. 1984. Family I. *Pseudomonadaceae*, p. 141. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Williams Co., Baltimore, Md.
31. Patel, N., S. L. Stenmark-Cox, and R. A. Jensen. 1978. Enzymological basis for reluctant auxotrophy for phenylalanine and tyrosine in *Pseudomonas aeruginosa*. J. Biol. Chem. **253**:2972–2978.
32. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
33. Pier, G. B., G. Meluleni, and J. B. Goldberg. 1995. Clearance of *Pseudomonas aeruginosa* from the murine gastrointestinal tract is effectively mediated by O-antigen-specific circulating antibodies. Infect. Immun. **63**:2818–2825.
34. Pier, G. B., and D. M. Thomas. 1982. Lipopolysaccharide and high molecular weight polysaccharide serotypes of *Pseudomonas aeruginosa*. J. Infect. Dis. **148**:217–223.
35. Ratnaningsih, E., S. Dharmsthiti, V. Krishnapillai, A. Morgan, M. Sinclair, and B. W. Holloway. 1990. A combined physical and genetic map of *Pseudomonas aeruginosa* PAO. J. Gen. Microbiol. **136**:2351–2357.
36. Reeves, P. 1993. Evolution of *Salmonella* O antigen variation by interspecific gene transfer on a large scale. Trends Genet. **9**:17–22.
37. Rivera, E., L. Vila, and J. Barbe. 1996. The *uvrB* gene of *Pseudomonas aeruginosa* is not DNA damage inducible. J. Bacteriol. **178**:5550–5554.
38. Rocchetta, H. L., L. L. Burrows, J. C. Pacan, and J. S. Lam. 1998. Three rhamnosyltransferases responsible for assembly of the A-band D-rhamnan polysaccharide in *Pseudomonas aeruginosa*: a fourth transferase, WbpL, is required for the initiation of both A-band and B-band lipopolysaccharide synthesis. Mol. Microbiol. **28**:1103–1119. (Erratum, **30**:1131, 1998.)
39. Sau, S., N. Shasin, E. R. Wann, J. C. Lee, T. J. Foster, and C. Y. Lee. 1997. The *Staphylococcus aureus* allelic genetic loci for serotype 5 and 8 capsule expression contain the type-specific genes flanked by common genes. Microbiology **143**:2395–2405.
40. Sawa, T., M. Ohara, K. Kurahashi, S. S. Twining, D. W. Frank, D. B. Doroques, T. Long, M. A. Gropper, and J. P. Wiener-Kronish. 1998. In vitro cellular toxicity predicts *Pseudomonas aeruginosa* virulence in lung infections. Infect. Immun. **66**:3242–3249.
41. Schweizer, H. P. 1991. *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19. Gene **97**:109–112.
42. Schweizer, H. P. 1993. Small broad-host-range gentamycin resistance gene cassettes for site-specific insertion and deletion mutagenesis. BioTechniques **15**:831–834.
43. Schweizer, H. P., and T. T. Hoang. 1995. An improved system for gene replacement and *xylE* fusion analysis in *Pseudomonas aeruginosa*. Gene **138**:15–22.
44. Shortridge, V. D., M. L. Pato, A. I. Vasil, and M. L. Vasil. 1991. Physical mapping of virulence-associated genes in *Pseudomonas aeruginosa* by transverse alternating-field electrophoresis. Infect. Immun. **59**:3596–3603.
45. Simon, R., U. Priefer, and A. Puhler. 1983. A broad-host-range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. Bio/Technology **1**:784–791.
46. Skurnik, M., R. Venho, P. Toivanen, and A. Al-Hendy. 1995. A novel locus of *Yersinia enterocolitica* serotype O:3 involved in lipopolysaccharide outer core biosynthesis. Mol. Microbiol. **17**:575–594.
47. Smith, A. W., and B. H. Iglewski. 1989. Transformation of *Pseudomonas aeruginosa* by electroporation. Nucleic Acids Res. **17**:10509.
48. Tassios, P. T., V. Gennimata, A. N. Maniatis, C. Fock, N. J. Legakis, and The Greek *Pseudomonas aeruginosa* Study Group. 1998. Emergence of multidrug resistance in ubiquitous and dominant *Pseudomonas aeruginosa* serogroup O:11. J. Clin. Microbiol. **36**:897–901.
49. West, S. E. H., and B. H. Iglewski. 1988. Codon usage in *Pseudomonas aeruginosa*. Nucleic Acids Res. **16**:9323–9335.
50. West, S. E. H., S. A. Kaye, A. N. Hamood, and B. H. Iglewski. 1994. Characterization of *Pseudomonas aeruginosa* mutants that are deficient in exotoxin A synthesis and are altered in expression of *regA*, a positive regulator of exotoxin A. Infect. Immun. **62**:897–903.
51. Woo, T. H. S., A. F. Cheng, and J. M. Ling. 1992. An application of a simple method for the preparation of bacterial DNA. BioTechniques **13**:696–698.