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O-Antigens of *Escherichia coli* Strains O81 and HS3-104 Are Structurally and Genetically Related, Except O-Antigen Glucosylation in *E. coli* HS3-104

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Abstract—Glycerophosphate-containing O-specific polysaccharides (OPSs) were obtained by mild acidic degradation of lipopolysaccharides isolated from *Escherichia coli* type strain O81 and *E. coli* strain HS3-104 from horse feces. The structures of both OPSs and of the oligosaccharide derived from the strain O81 OPS by treatment with 48% HF were studied by monosaccharide analysis and one- and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. Both OPSs had similar structures and differed only in the presence of a side-chain glucose residue in the strain HS3-104 OPS. The genes and the organization of the O-antigen biosynthesis gene cluster in both strains are almost identical with the exception of the *gtr* gene cluster responsible for glucosylations in the strain HS3-104, which is located elsewhere in the genome.

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Escherichia coli is a dominant facultative anaerobe of the gut microbiome in many mammals, including humans. Gut microbiome also contains numerous conditionally pathogenic strains [1]. The O-polysaccharide (O-antigen) is a component of the cell wall outer membrane lipopolysaccharide (LPS) in Gram-negative bacteria. It consists of oligosaccharide repeats (O-units) containing two to eight residues of common or rarely occurring sugars and their derivatives. The O-antigen is one of the most variable cell constituents, with variation in the types of sugars present, their arrangement within the O-unit, and the type of bonds in and between the O-units, thus providing the basis for serotyping of bacteria [2]. Being exposed on the cell surface, the O-antigen is a subject of intense selective pressure by the host immune system and bacteriophages [3].

Genes involved in the O-antigen biosynthesis in *E. coli* and some related bacteria are usually clustered in a chromosomal locus located between two conserved genes, *galF* and *gnd* [4]. Most of these genes fall into one of the three major classes: genes for the synthesis of nucleotide-activated sugars, genes for glycosyl transferases involved in the formation of bonds between the sugars, and O-unit processing genes [5].

More than 180 O-serogroups of *E. coli* are internationally acknowledged (http://www.ssidiagnostica.com/Products/Bacterial-strains/E-coli). Their O-antigen gene clusters have been sequenced [6], and >140 O-polysaccharide (OPS) structures have been determined

Abbreviations: bp, base pair; COSY, correlation spectroscopy; GC, gas chromatography; Gro, glycerol; HMBC, heteronuclear multiple-bond correlation; HSQC, heteronuclear single quantum correlation; OPS, O-specific polysaccharide; ROESY, rotating-frame Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; Und-P, undecaprenyl phosphate.

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(http://nevyn.organ.su.se/ECODAB/; http://csdb.glycoscience.ru/bacterial/). The goal of our investigation is the development of a chemical basis for classification of *E. coli* strains and molecular diagnostics of infectious diseases caused by these bacteria. For this purpose, it is necessary to elucidate the structures of OPSs in type strains of all *E. coli* O-serogroups and to annotate genes in the O-antigen gene clusters of these strains, taking into account the OPS structures determined.

In this work, we deciphered the structures of the OPSs from the earlier unstudied *E. coli* serogroup O81 and of the structurally related OPS from the *E. coli* strain HS3-104. The O81-antigen gene cluster was present in both strains and was consistent with the *E. coli* O81 OPS structure also typical for the OPS main chain in the HS3-104 strain. OPS from the HS3-104 strain is modified by the side-chain glucosylation; enzymes catalyzing this process are located outside of the O-antigen gene cluster.

MATERIALS AND METHODS

Bacterial strains, cell cultivation, and isolation of lipopolysaccharides. *Escherichia coli* O81 type strain (laboratory stock G1217) was from the Institute of Medical and Veterinary Science, Adelaide, Australia. *Escherichia coli* HS3-104 strain was isolated from horse feces [7]. The bacterial cells were grown to the late log phase in 8 liters of Luria–Bertani broth (pH 7.0) with constant aeration at 37°C and then washed and dried as described in [8]. The cells were extracted by the phenol–water method [9]; LPS preparations were obtained as described earlier [10].

OPS isolation. LPSs were hydrolyzed with 2% acetic acid aqueous solution at 100°C for 2 h; precipitated lipids were removed by centrifugation (13,000*g*, 20 min). Carbohydrates were fractionated by gel filtration on a Sephadex G-50 Superfine column (56 × 2.6 cm; Amersham Biosciences, Sweden) in 0.05 M pyridinium acetate buffer (pH 4.5); elution was monitored with a differential refractometer (Knauer, Germany). The OPS yield was 5 and 6% of total LPS mass for *E. coli* strains O81 and HS3-104, respectively.

Dephosphorylation. OPS from strain O81 (10 mg) was treated with aqueous 48% HF at 4°C for 24 h. HF was removed by a stream of air; the residue was dried over P_2O_5 and fractionated by gel filtration on a Fractogel TSK HW 40S column (85 × 1.6 cm) in 1% acetic acid to obtain oligosaccharide **1** (1.4 mg).

Monosaccharide analysis. Alditol acetates [11] were obtained by OPS hydrolysis with 2 M trifluoroacetic acid (120°C, 2 h) and analyzed by gas chromatography (GC) on an HP-5 capillary column with an Agilent 7820 GC system using a temperature gradient from 160°C (1 min) to 290°C at 7°C/min.

NMR spectroscopy. Samples were lyophilized from 99.9% D_2O and examined as solutions in 99.95% D_2O. ^{13}C NMR spectra were recorded with a Bruker DRX-500 instrument; other spectra were recorded with a Bruker Avance II 600 MHz spectrometer at 30°C (oligosaccharide **1** and O81 OPS) or at 60°C (HS3-104 OPS) using sodium 3-(trimethylsilyl)propanoate-2,2,3,3-d_4 (δ_H 0, δ_C –1.6) as an internal standard for calibration. Two-dimensional NMR spectra were obtained using standard Bruker software and the Bruker TopSpin 2.1 program for acquiring and processing of NMR data. The spin-lock time of 60 ms and the mixing time of 150 ms were used in the two-dimensional $^1H,^1H$ TOCSY and $^1H,^1H$ ROESY experiments, respectively. A 60-ms delay was used for evolution of long-range coupling to optimize $^1H,^{13}C$ HMBC spectroscopy experiments.

Sequencing and analysis of genes. Genomic DNA from *E. coli* HS3-104 cells was isolated with a Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. Extracted DNA (100 ng) was disrupted into 200-300-bp fragments by sonication using a Covaris S220 System (Covaris, USA). The shotgun library was prepared with an Ion Xpress™ Plus Fragment Library Kit (Life Technologies, USA). Emulsion PCR was performed using an Ion PGM™ Template OT2 200 Kit (Life Technologies). DNA sequencing was performed using an Ion Torrent PGM (Life Technologies) with an Ion 318 chip using Ion PGM™ Sequencing 200 Kit v2 (Life Technologies). The genome sequence was assembled using the Newbler v.3.0 program with standard parameters. To correct Ion Torrent homopolymer errors that could result in assembly errors [12] and artificial frame shifts in coding sequences, the HomoHomo tool was applied (www.github.com/paraslonic/HomoHomo) [13]. The corrected draft of the genome sequence was annotated using the PROKKA 1.7 program [14]. Sequences of the contigs obtained by the whole-genome sequencing were deposited at the DDBJ/ENA/GenBank under accession No. PHFI00000000.

Suggested gene functions were assigned using the NCBI BLAST program to screen homologous sequences in the GenBank database. Conserved domains in each protein were annotated by comparing them to conserved protein motifs from the Pfam database (pfam.sanger.ac.uk). The TMHMM analysis program v.2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) was used to identify potential transmembrane domains. The functions of glycosyl transferase genes were predicted by comparing to genes of other *E. coli* O-serogroups with the same glycoside bonds in their OPSs.

RESULTS AND DISCUSSION

Structure of O-specific polysaccharides. OPSs were obtained by mild acidic degradation of LPSs isolated

from bacterial cells by phenol–water extraction and then separated from low-molecular-mass components by gel filtration on a Sephadex G-50 column. GC analysis of alditol acetates derived after full acidic hydrolysis of the O81 and HS3-104 OPSs identified Glc, Gal and GalNAc at the ratios of 1.5 : 1.0 : 1.3 and 2.5 : 1.0 : 1.3, respectively.

The ^{31}P NMR spectrum of the O81 OPS contained a signal for a monophosphate group at δ 0.23. Assignment of the ^{1}H NMR and ^{13}C NMR spectra of the O81 OPS (Fig. 1) was complicated, most likely, due to non-stoichiometric phosphorylation. For this reason, the OPS was dephosphorylated by treatment with 48% aqueous HF. Dephosphorylation was accompanied by cleavage of the β-Gal*p*NAc-(1→6)-Gal*p*NAc bond (Fig. 2). The resulting oligosaccharide **1** was studied by one- and two-dimensional NMR spectroscopies (for ^{1}H and ^{13}C NMR signal assignment in oligosaccharide **1** see Table 1). It was found that oligosaccharide **1** contained the same monosaccharides as the initial OPS. The structure of oligosaccharide **1** (Fig. 2) was established using two-dimensional ^{1}H,^{1}H ROESY and ^{1}H,^{13}C HMBC spectroscopy (Table 2).

The data obtained for oligosaccharide **1** enabled assignment of the major series of signals in the ^{1}H and ^{13}C NMR spectra of the O81 OPS (Table 1). Comparison of ^{13}C NMR chemical shifts for these two compounds showed that the GalNAc residue (**A**) at the reducing end of oligosaccharide **1** is β-linked in the OPS. The correlation between C-1 of unit **A** and H-6b of another GalNAc residue (**D**) at δ 102.6/3.99 indicated that the repeating units are connected by the β-Gal*p*NAc-(1→6)-Gal*p*NAc (**A**→**D**) bonds.

Beside monosaccharides, the OPS also contained glycerol 1-phosphate bound to the third GalNAc residue (unit **C**) at position 3, as follows from the correlation between the phosphate group signal and the GalNAc (unit **C**) H-3 and glycerol H-1 at δ 0.23/4.23 and 0.23/3.87, respectively, in the two-dimensional ^{1}H,^{31}P HMBC spectrum. Therefore, the O81 OPS has the structure shown in Fig. 2. Most likely, the minor series of signals in the OPS O81 spectrum belongs to the nonphosphorylated polysaccharide, but assignment of its NMR signals was complicated by multiple coincidences with signals of the major series.

Similar analysis of the NMR spectra allowed elucidation of the structure of the HS3-104 strain OPS (for assigned ^{1}H NMR and ^{13}C NMR (Fig. 1) signals see Table 1). The HS3-104 OPS structure is similar to that of the O81 OPS and differs only in the presence of an additional side-chain α-Glc*p* residue (unit **F**). The two-dimensional ROESY spectrum showed a correlation between the unit **F** H-1 and unit **B** H-2; therefore Glc (unit **F**) is attached to Glc (unit **B**) at position 2. The sequence of other constituent monosaccharides and the bonds between them were the same as in the O81 OPS;

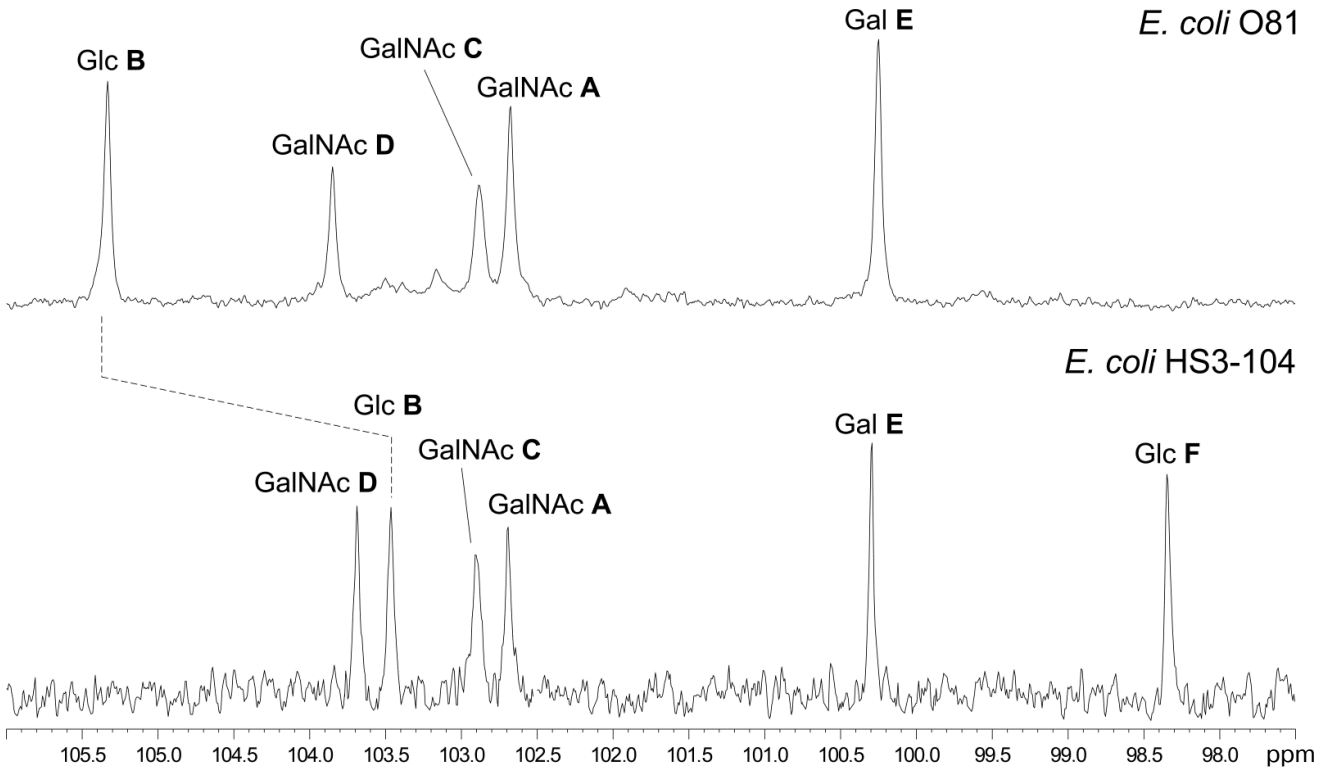
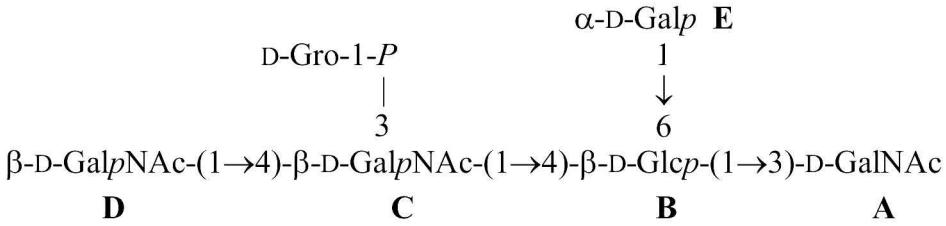
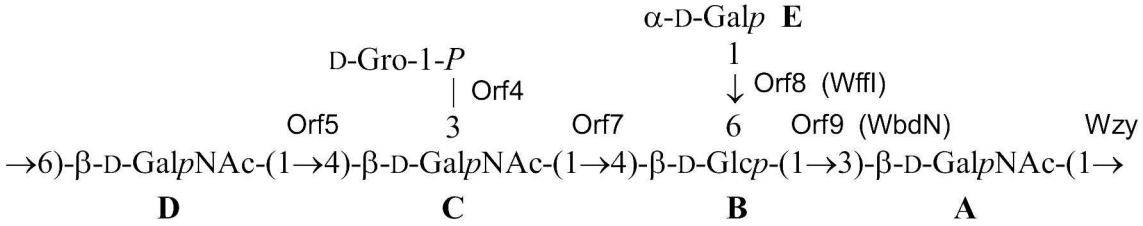


Fig. 1. Region of anomeric carbons in the ^{13}C NMR spectra of OPSs from *E. coli* strains O81 (top) and HS3-104 (bottom).

Oligosaccharide **1**



E. coli O81 OPS



E. coli HS3-104 OPS

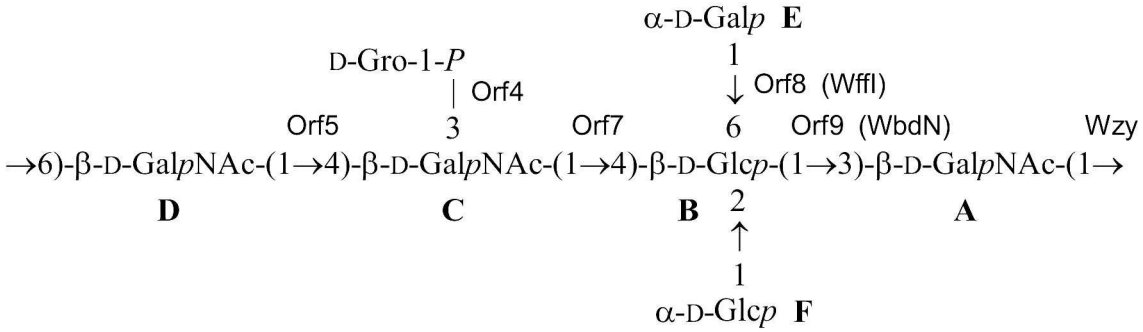


Fig. 2. Structures of *E. coli* OPSs and oligosaccharide **1**. *Escherichia coli* O81 transferases are indicated next to the bonds they were assigned to. Homologs of Orf8 and Orf9 with known functions are given in parentheses.

hence, the HS3-104 OPS has the structure shown in Fig. 2.

The OPSs studied are distinguished in the presence of a side-chain glycerol 1-phosphate. Phosphorylated OPSs are not uncommon for *E. coli*, including those containing glycerol 1-phosphate that can be either attached as a side chain (serogroups O37, O81, O100) or incorporated into the main chain (serogroups O28ab, O28ac, O29, O42) (http://nevyn.organ.su.se/ECODAB/; http://csdb.glycoscience.ru/bacterial/). Glycerol 2-phosphate or D-glyceric acid 2-phosphate are present in the OPSs of *E. coli* O130 and O82, respectively. In some other *E. coli* OPSs, phosphate links ribitol and a monosaccharide (serogroups O118 and O151) or two monosaccharides in the main chain (serogroups O84, O152, O160, O172, O173, O181) (http://nevyn.organ.su.se/ECODAB/; http://csdb.glycoscience.ru/bacterial/).

Characterization of the O81 antigen gene cluster. The O-antigen gene cluster of *E. coli* O81 has been sequenced earlier [6]. It contains nine genes transcribed in the direction from *galF* to *gnd* (Fig. 3). Descriptions of all open reading frames (*orf*s) and their homologs found by bioinformatic analysis are given in Table 3. No genes for the synthesis of nucleotide sugar precursors of Glc and Gal were found between *galF* and *gnd* in *E. coli* O81, as they are usually located outside the O-antigen gene cluster [15].

Either GlcNAc or GalNAc is the first sugar in the O-unit of most *E. coli* strains. The *wecA* (*rfe*) gene is responsible for the transfer of D-GlcNAc-*P* from UDP-D-GlcNAc to undecaprenyl phosphate (Und-P) with the formation of UndPP-D-GlcNAc. This gene is located in the cluster responsible for the synthesis of enterobacterial common antigen [16]. When the first sugar is GalNAc (as

Table 1. ^{1}H and ^{13}C NMR chemical shifts (δ, ppm) measured at 30°C

Monosaccharide	C-1 *H-1*	C-2 *H-2*	C-3 *H-3*	C-4 *H-4*	C-5 *H-5*	C-6 *H-6 (6a, 6b)*
OPS from *E. coli* O81						
→3)-β-D-Gal*p*NAc-(1→ **A**	102.6 *4.56*	52.5 *3.99*	81.7 *3.88*	69.4 *4.09*	76.1 *3.67*	62.4 *3.80*
→4,6)-β-D-Glc*p*-(1→ **B**	105.1 *4.50*	73.8 *3.32*	75.7 *3.63*	80.9 *3.56*	74.8 *3.68*	67.8 *3.74; 3.77*
→3,4)-β-D-Gal*p*NAc-(1→ **C**	102.9 *4.52*	53.2 *3.94*	75.9 *4.23*	75.7 *4.20*	75.6 *3.71*	62.3 *3.71; 3.80*
→6)-β-D-Gal*p*NAc-(1→ **D**	103.8 *4.66*	53.6 *3.97*	72.8 *3.68*	69.1 *3.90*	74.3 *3.75*	69.3 *3.84; 3.99*
α-D-Gal*p*-(1→ **E**	100.3 *4.97*	69.7 *3.83*	70.8 *3.81*	70.7 *3.99*	72.7 *3.87*	62.6 *3.76*
D-Gro-1-*P*	67.8 *3.86; 3.89*	72.2 *3.89*	63.7 *3.60; 3.65*			
Oligosaccharide **1**						
→3)-α-D-Gal*p*NAc **Aα**	92.4 *5.20*	50.1 *4.28*	78.6 *4.03*	69.9 *4.16*	71.4 *4.11*	62.0 *3.80; 3.83*
→3)-β-D-Gal*p*NAc **Aβ**	96.3 *4.68*	53.5 *3.97*	81.9 *3.85*	69.1 *3.90*	76.2 *3.64*	62.0 *3.80; 3.83*
→4,6)-β-D-Glc*p*-(1→ **B**	104.9[a] *4.57*[a]	73.6 *3.31*	75.4 *3.64*	80.5 *3.57*	74.7 *3.69*	67.7 *3.72; 3.78*
→3,4)-β-D-Gal*p*NAc-(1→ **C**	103.0 *4.48*	53.8 *3.87*	72.0 *3.84*	76.0 *4.12*	75.8 *3.69*	62.2 *3.71; 3.82*
β-D-Gal*p*NAc-(1→ **D**	103.5 *4.66*	53.9 *3.88*	72.2 *3.74*	69.2 *4.10*	75.9 *3.67*	62.3 *3.78*
α-D-Gal*p*-(1→ **E**	100.1 *4.98*	69.3 *3.82*	70.7 *3.82*	70.5 *3.97*	72.4 *3.88*	62.4 *3.76*
OPS from *E. coli* HS3-104						
→3)-β-D-Gal*p*NAc-(1→ **A**	102.3 *4.58*	52.3 *4.01*	80.7 *3.97*	68.5 *4.16*	75.6 *3.70*	[b]
→2,4,6)-β-D-Glc*p*-(1→ **B**	103.4 *4.74*	77.2 *3.53*	74.3 *3.77*	81.0 *3.58*	74.5 *3.76*	67.9 *3.72; 3.80*
→3,4)-β-D-Gal*p*NAc-(1→ **C**	102.8 *4.54*	53.2 *3.96*	75.8 *4.25*	75.6 *4.20*	75.6 *3.70*	[b]
→6)-β-D-Gal*p*NAc-(1→ **D**	103.8 *4.67*	53.5 *3.98*	72.4 *3.71*	69.1 *3.91*	74.4 *3.76*	68.8 *4.00*
α-D-Gal*p*-(1→ **E**	100.3 *4.98*	70.6 *3.85*	70.8 *3.85*	70.7 *4.01*	72.1 *3.90*	[b]
α-D-Glc*p*-(1→ **F**	98.2 *5.23*	73.1 *3.49*	74.5 *3.76*	70.9 *3.44*	73.0 *4.06*	61.7 *3.76; 3.79*
D-Gro-1-*P*	67.7 *3.89*	72.1 *3.88*	63.6 *3.62; 3.68*			

Note: ^{1}H NMR chemical shifts are italicized. Chemical shifts for N-acetyl groups: δ_H 2.03-2.08; δ_C 23.7-23.9 (Me), 175.2-176.4 (CO).

[a] When linked to **Bα**; δ_C = 105.1 and δ_H = 4.51 when linked to **Bβ**.

[b] Chemical shifts are δ_C 62.3-62.5 and δ_H 3.74-3.88.

Table 2. Correlations for H-1 and C-1 in the two-dimensional $^{1}H,^{1}H$ ROESY and $^{1}H,^{13}C$ HMBC spectra of oligosaccharide **1** from *E. coli* O81

Atom in monosaccharide residue (δ)	Correlations to atom in monosaccharide residue (δ)	
	$^{1}H,^{1}H$ ROESY	$^{1}H,^{13}C$ HMBC
B(α) H-1 (4.57)	**A**α H-3 (4.03), **B** H-5 (3.69)	**A**α C-3 (78.6)
B(β) H-1 (4.51)	**A**β H-3 (3.85), **B** H-5 (3.69)	**A**β C-3 (81.9)
C H-1 (4.48)	**B** H-4 (3.57), **C** H-5 (3.69)	**B** C-4 (80.5)
C C-1 (103.0)		**B** H-4 (3.57)
D H-1 (4.66)	**C** H-4 (4.12), **D** H-5 (3.67)	**C** C-4 (76.0)
D C-1 (103.5)		**C** H-4 (4.12)
E H-1 (4.98)	**B** H-5 (3.69), **B** H-6a, 6b (3.72, 3.78), **E** H-2, H-3 (3.82)	**B** C-6 (67.7), **E** C-3 (70.7), **E** C-5 (72.4)
E C-1 (100.1)		**B** H-6a, H-6b (3.72, 3.78)

in *E. coli* O81), 4-epimerase Gnu converts UndPP-D-GlcNAc into UndPP-D-GalNAc [17], and the *gnu* gene is located immediately upstream of the *galF* gene [18]. In *E. coli* O81, this gene has been identified and named *gne* [6]. However, the name Gne has been already used for another 4-epimerase that converts UDP-D-GlcNAc to UDP-D-GalNAc [18]; therefore, we recommend renaming the gene responsible for the synthesis of UndPP-D-GalNAc as *gnu*. There are two more D-GalNAc residues in the O81-O unit. *orf1* was identified as a gene coding for 4-epimerase involved in the synthesis of UDP-D-GalNAc and named *gne* accordingly.

orf2 was annotated as glycerol-3-phosphate cytidyltransferase gene; we presume that its protein product might be involved in the formation of D-Gro-1-*P*. However, this suggestion requires experimental confirmation.

There are five transferase genes in the O81 O-antigen gene cluster, that were annotated taking into account the established OPS structure. *orf4* was identified as a glycerophosphotransferase gene that encodes the enzyme that supposedly transfers D-Gro-1-*P* to one of the D-GalNAc residues.

*orf*s *5*, *7*, *8*, and *9* were annotated as glycosyltransferase genes (Figs. 3 and 4). WbdN, a UDP-Glc:GalNAc-diphosphate-lipid β1,3-Glc-transferase, has been found to catalyze formation of the β-D-Glc*p*-(1→3)-D-Gal*p*NAc bond in *E. coli* O157 [19]. Since Orf9 of *E. coli* O81 shares 48% identity with WbdN from *E. coli* O157 and O-antigens of these two strains have the β-D-Glc*p*-(1→3)-D-Gal*p*NAc disaccharide fragment in common, we propose that Orf9 is a homolog of WbdN responsible for the synthesis of this fragment. α-D-Gal*p*-(1→6)-D-Glc*p* is the only identical bond in the O-antigens of *E. coli* strains O130 [20] and O81. Orf8 of *E. coli* O81 is 36% identical to WffI of *E. coli* O130 [15]; therefore, these two enzymes might have the same role in the formation of the α-D-Gal*p*-(1→6)-D-Glc*p* bond. Hence, *orf5* and *orf7* are β-D-Gal*p*NAc-transferases that catalyze formation of the β-D-Gal*p*NAc-(1→4)-D-Gal*p*NAc and β-D-Gal*p*NAc-(1→6)-D-Glc*p* bonds. No detailed assignment has not been done for these *orf*s due to the lack of their homologs with known functions in available databases.

orf3 and *orf6* were identified as *wzx* and *wzy* genes for the O-unit flippase and O-antigen polymerase, respectively. The presence of these genes indicated that synthesis and translocation of the O81 O-antigen occurs by the Wzx/Wzy-dependent mechanism that includes assembly of the O-unit on a lipid carrier at the inner membrane cytoplasmic side followed by the O-unit translocation to the periplasmic side.

The genome of *E. coli* HS3-104 has been sequenced. Its O-antigen gene cluster is essentially identical to that of

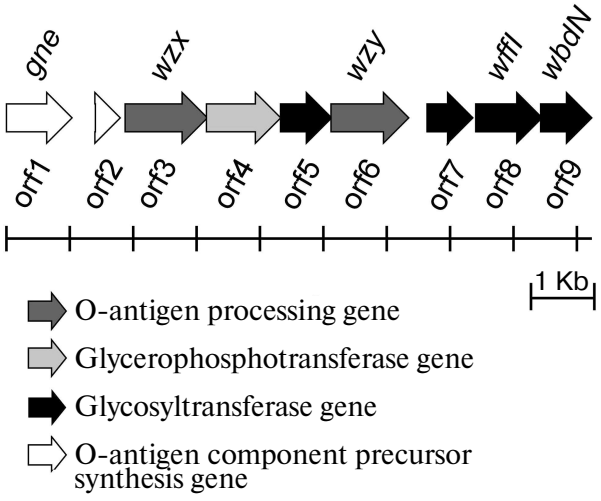


Fig. 3. Organization of *E. coli* O81 O-antigen gene cluster (adapted on the basis of Iguchi et al. data [6]). Homologs of *orf8* and *orf9* with known functions are designated.

Table 3. Characteristics of *orf*s in the O81 antigen gene cluster

Gene	Position of gene	G+C content, %	Conserved domain(s)	Similar protein(s), strain(s) (Genbank accession No.)	% identical/ % similar (total number of amino acids)	Putative function of protein
orf1	3827...4864	32.85	GDP-mannose 4,6-dehydratase family (PF16363) E value = 1.7e-54	UDP-glucose 4-epimerase [*Escherichia coli*] (WP_096159385.1)	99/99 (345)	UDP-glucose 4-epimerase
orf2	5230...5625	34.59	cytidylyltransferase-like family (PF01467) E value = 7.4e-20	glycerol-3-phosphate cytidylyltransferase [*Escherichia coli*] (WP_024193472.1)	79/93 (131)	glycerol-3-phosphate cytidylyltransferase
orf3	5698...6999	59.56		Wzx [*Shigella dysenteriae*] (ACD37113.1)	34/58 (433)	O-antigen flippase
orf4	6983...8155	28.9	CDP-glycerol: poly(glycerophosphate) glycerophosphotransferase family (PF04464) E value = 5.4e-57	CDP-glycerol glycerophosphotransferase family protein [*Proteus mirabilis*] (WP_049256327.1)	46/62 (390)	putative glycerophosphotransferase
orf5	8152...8958	29.73	glycosyltransferase family 2 (PF00535) E value = 2.3e-7	glycosyltransferase [*Escherichia coli*] (WP_014640169.1)	45/63 (268)	glycosyltransferase
orf6	8948...10177	27.72	EspG family (PF14897) E value = 1.9e-8	O38 family O-antigen polymerase [*Escherichia coli*] (WP_053291797.1)	24/46 (409)	O-antigen polymerase
orf7	10465...11187	30.42	glycosyltransferase family 2 (PF00535) E value = 8e-9	glycosyltransferase family 2 protein [*Photobacterium aquimaris*] (WP_065190093.1)	53/68 (240)	putative glycosyltransferase
orf8	11232...12278	34.19	glycosyltransferase group 1 family (PF00534) E value = 5.1e-32	WffI [*Escherichia coli*] (ACD37154)	36/56 (348)	glycosyltransferase
orf9	12257...13063	31.97	glycosyl transferase family 2 (PF00535) E value = 1.2e-33	putative glycosyltransferase WbdN [*Escherichia coli*] (AAC32339)	48/65 (268)	glycosyltransferase

E. coli O81 (>99% nucleotide identity). Side-chain glucosylation in the OPS of *E. coli* HS3-104 is evidently driven by the *gtrABC* genes found on a 3145-bp contig, for which such function has been well established [21, 22]. Encoded GtrA, GtrB, and GtrC are identical to the corresponding proteins of *E. coli* KCJK4935 (GenBank accession numbers PBU28851, PBU28852, and PBU28853) by 98, 100, and 99%, respectively. Undecaprenol glucosyltransferase GtrB (synthesis of UndP-D-Glc from UDP-D-Glc and UndP) and flippase GtrA (translocation of UndP-D-Glc through the cytoplasmic membrane) are highly conserved proteins, whereas glucosyltransferase GtrC, that links a glucosyl group to the O-antigen backbone, is serotype-specific. The *gtr* gene cluster is present in many enteric bacteria and usually has a bacteriophage origin [21-23].

In conclusion, the content and assigned functions of the genes involved in the O-antigen synthesis in the studied *E. coli* strains are consistent with the OPS structures established.

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