RESEARCH ARTICLE

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The first sugar of the repeat units is essential for the Wzy polymerase activity and elongation of the O-antigen lipopolysaccharide

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Aim: In the Wzx/Wzy-dependent assembled pathway, the assembled O-antigen repeat units are translocated by a Wzx translocase and then, polymerized by the integral membrane protein Wzy to form a glycan chain. In this study, we investigate the relation between the first sugar of the O-antigen repeat unit and the Wzy polymerase. **Material & methods:** Cross-complementation studies in two different reducing/non-reducing end affinity systems were performed. **Results & conclusion:** We demonstrate that the activity of the *Escherichia coli* O-antigen polymerase (Wzy) is dependent on the first sugar of the O-antigen repeat unit to produce the O-antigen polymerization and therefore, there is a need for a concerted action with the enzyme transferring the initial HexNAc to undecaprenol phosphate. Furthermore, polymerization activity of *Aeromonas hydrophila* Wzy-O34 is permissive with the sugar at the non-reducing end of the O-antigen repeat unit.

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• *Escherichia coli* • O-antigen polymerization • Wzy O-antigen assembly pathway

Bacterial cell surface polysaccharides play important roles in processes critical for bacterial pathogenicity and environmental adaptations. Lipopolysaccharide (LPS) is the major glycoconjugate constituent of the outer leaflet of the outer membrane (OM), in Gram-negative bacteria. LPS molecules comprise three chemically distinct regions: lipid A, a hydrophobic glycolipid which anchors LPS in the bacterial membrane; core oligosaccharide (OS), a nonrepeating oligosaccharide which usually contains heptoses and keto-deoxyoctulosonic acid; and the O-specific polysaccharide (O-antigen), a glycan chain of homo- or hetero-polysaccharide repeat units [1]. In general, variations of lipid A and core oligosaccharide are relatively limited. However, the chemical composition and structure of O-antigens vary widely among Gram-negative bacteria, leading to a large number of O-serotypes [2]. For example, there are over 186 O-antigen forms for *Escherichia coli* [3].

Four major modes of O-antigen assembly have been defined: the Wzm/Wzt-, the Wzx/Wzy-, the synthetase- and the Wzk-dependent pathways [4]. Nearly all *E. coli* O-antigens are synthesized by the Wzx/Wzy-dependent pathway [5], as is in *Aeromonas hydrophila* O34 [6]. This pathway starts at the cytosolic face of the inner membrane by the formation of a linkage between the lipid carrier undecaprenyl phosphate (Und-P) and the first sugar 1-phosphate, of the corresponding O-antigen unit, transferred from a sugar nucleoside diphosphate. Two different classes of integral membrane protein are able to transfer the first sugar onto Und-P to give Und-PP-linked sugar: the *N*-acetylhexosamine-1-phosphate transferase and the polyisoprenyl-phosphate hexose-1-phosphate transferase. In *E. coli*, the initial transferase is usually WecA, which transfers *N*-acetylglucosamine-phosphate (GlcNAc-1-P)

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to Und-P to give Und-PP-linked GlcNAc [5]. In *A. hydrophila* AH-3, the initial transferase is WecP, which transfers *N*-acetylgalactosamine-phosphate (GalNAc-1-P) to Und-P to give Und-PP-linked GalNAc [7]. The remaining sugars of the O-antigen repeat unit are subsequently transferred from nucleotide-sugar precursors to the Und-PP-linked sugar by a serial action of glycosyltransferases. Completed O units are flipped across the inner membrane by the integral membrane protein Wzx [8,9] and then assembled on the periplasmic side of the inner membrane by the Wzy O-antigen polymerase [10]. Wzx and Wzy both exhibit enormous sequence diversity. The chain length of the final polymer is regulated by the O-antigen chain length regulator Wzz [11]. Finally, the O-antigen polymer is ligated to the lipid A-core OS by an O-antigen ligase (WaaL) [12] and complete LPS molecule is translocated from the inner membrane to the outer leaflet of the outer membrane by the Lpt proteins [13].

The O-antigen polymerization reaction generates a glycosidic linkage between the nonreducing end of a new O-antigen unit linked to Und-PP, to the reducing end of the Und-PP-linked growing polymer [14,15]. Wzy O-antigen polymerases are very hydrophobic integral membrane proteins that contain 10-14 transmembrane domains with two major periplasmic loops. Even at the amino acid level, they show little similarity to each other in terms of primary sequence, making the identification of catalytic and binding residues difficult [14]. The polymerase genes are located in the gene clusters involved in the polysaccharide biosynthesis that polymerize.

The O-antigen units of *E. coli* O7, O150 and O159, as well as *A. hydrophila* O34, are synthesized individually at the cytosolic side of the inner membrane by the Wzx/Wzy-dependent assembly pathway. Each repeat unit is translocated across the cytoplasmic membrane by the Wzx (O-unit flippase) and then, it needs to be polymerized. The O-antigen polymerase (Wzy)

Table 1. Bacterial strains and plasmids used.

Strains or plasmids	Relevant characteristics	Source or ref.
Escherichia coli *strains*		
DH5α	F$^-$*end A hsdR17* (rK$^-$ mK$^+$) *supE44 thi-1 recA1 gyr-A96_80lacZ*M15	[17]
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17supE44 relA1 lac* (F$^-$*proAB lacI*q*Z*_M15 Tn*10*)	Stratagene
VW187	O7:K1; clinical isolate	[18]
MV501	VW187; *wecA*::Tn*10* Tc^R	[18]
O150	O25b-ST131 (serotype O150)	[19]
O159	CCUG36899 (serotype O159)	[19]
O150Δ*wecA*	O25b-ST131 *wecA* mutant in frame with pKO3	This study
O159Δ*wecA*	CCUG36899 *wecA* mutant in frame with pKO3	This study
Aeromonas *strains*		
AH-405	AH-3, spontaneous Rif^R	[20]
AH-405Δ*wzy*	AH-405 *wzy* mutant in frame with pDM4	[6]
Plasmids		
pRK2073	Helper plasmid, Spc^R	[6]
pKO3	Cm^R*sacB* temperature sensitive suicide vector	[21]
pKO3Δ$wecA_{O150}$	pKO3 with *wecA* in frame deletion	This study
pGEMT-Gne_{Ah}	pGEM-T vector with complete gne of AH-3	[22]
pBAD33	Arabinose inducible expression vector, Cm^R	ATCC
pBAD33-Gm	pBAD33 vector with Gm^R	[23]
pBAD33-$WecP_{Ah}$	pBAD33-Gm with *Aeromonas hydrophila* AH-3 *wecP*	[7]
pBAD33-$WecA_{Ec}$	pBAD33-Gm with *Escherichia coli* VW187 *wecA*	[7]
pBAD-WecP-Wzy_{Ah}	pBAD33-$WecP_{Ah}$ with *A. hydrophila* AH-3 *wzy*	[24]
pBAD-WecA-$WzyE_{Ec}$	pBAD33-$WecA_{Ec}$ with *E. coli* DH5α wzy enterobacterial common antigen	[24]
pBAD-$WzyE_{Ec}$	pBAD33 with *E. coli* DH5α *wzy* enterobacterial common antigen	[24]

transfers the growing chain to the nonreducing end of the new O-subunit, establishing a glycosidic bond. The concerted action of Wzy and Wzz (O-antigen chain length regulator) is responsible for the polymerization of the O-antigen units to a certain length distribution that is unique to each O-antigen [16]. In the current study, we show that *E. coli* O-antigen polymerase has specificity for the first sugar of the O-antigen repeat unit and, therefore, the concerted action of the enzyme mediating the transfer of HexNAc to Und-P and the O-antigen polymerase (Wzy) is involved in the O-antigen polymerization by the Wzx/Wzy-dependent O-antigen export and assembly pathway. Furthermore, *A. hydrophila* Wzy-O34 polymerization activity is permissive with the sugar at the nonreducing end of the O-antigen repeat unit.

Materials & methods

• Bacterial strains, plasmids & growth conditions

Bacterial strains and plasmids used are shown in **Table 1**. *Escherichia coli* were grown either in Miller lysogeny broth (LB) and LB Miller agar. *Aeromonas* were grown either in tryptic soy broth (TSB) or tryptic soy agar (TSA). Chloramphenicol (25 μg/ml), gentamicin (20 μg/ml) or ampicillin (100 μg/ml) were added to the different media when required.

• General DNA methods

Standard DNA manipulations were done as described [25]. DNA restriction endonucleases, T4 DNA ligase, *E. coli* DNA polymerase (Klenow fragment) and alkaline phosphatase were used as recommended by the suppliers.

• DNA sequencing & computer analysis of sequence data

Double-stranded DNA sequencing was performed by using the dideoxy-chain termination method [26] with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Oligonucleotides used for genomic DNA amplifications and DNA sequencing were designed from GenBank EU274663.1, DQ119103.1, CP003034.1, NC_007779.1 or AP009378, and purchased from Sigma–Aldrich. Deduced amino acid sequences were compared with those of DNA translated in all six frames from nonredundant GenBank and EMBL databases by using the BLAST [27] network service at the National Center for Biotechnology Information and European Biotechnology Information. ClustalW was used for multiple-sequence alignments [28] and the topology maps were predicted by using TMMTOP 2.0 [29].

• Mutant construction

Escherichia coli O150Δ*wecA* and O159Δ*wecA* mutants were constructed by creating an *in vitro* in-frame deletion of the gene [21], as previously described [30]. *Escherichia coli* O25b-ST131 (O150) and CCUG 36899 (O159) strains were used [19]. The mutant was constructed using *E. coli* O150 chromosomal DNA and primers Ecrfe-A2wecA-A(5′-GGAAGATCTGTGTTG-ACCGGTGGTGTGG-3′), Ecrfe-BwecA-B (5′-CCCATCCACTAAACTTAAACACT GGTGACGTTTGCGGAAGT-3′), Ecrfe-CwecA-C (5′-TGTTTAAGTTTAG TGGATG GGGGTGGCAGCCCCAATTTAAC -3′), and Ecrfe-D2wecA-D (5′-GGAAGA TCTATCCGGCGGTCATAGATGG-3′) in two sets of asymmetric PCRs to amplify DNA fragments AB and CD, respectively. DNA fragments AB and CD were annealed at their overlapping region (underlined letters in primers B and C) and amplified by PCR as a single fragment, using primers A and D. The fusion product was purified, *Bgl*II-digested (double-underlined letters in primers A and D), ligated into *Bam*HI-digested and phosphatase-treated pKO3 vector, electroporated into *E. coli* DH5-α, and plated on chloramphenicol LB agar plates at 30°C to obtain plasmid pKO3 Δ $wecA_{O150}$. The mutated gene was transferred to the chromosome by homologous recombination using the temperature-sensitive suicide plasmid pKO3, containing the counterselectable marker *sacB*. The plasmid containing the engineered in-frame deletion (pKO3Δ$wecA_{O150}$) was transferred into *E. coli* O150 and O159 strains by transformation. Mutants were independently selected based on growth on LB agar containing 10–15% sucrose and loss of the chloramphenicol resistance marker of vector pKO3 [21]. The mutations in each strain were confirmed by sequencing of the whole constructs in amplified PCR products.

• Complementation studies

Plasmids pBAD33-$WecP_{Ah}$, pBAD33-$WecA_{Ec}$ or pBAD-WecP-Wzy_{Ah} were independently transferred into the *E. coli wecA* mutants by electroporation. Plasmid pBAD-$WzyE_{Ec}$ was transferred into the *A. hydrophila* AH-3 *wzy* mutant by triparental mating using the mobilizing strain HB101/pRK20173. Transformants were selected on plates containing gentamycin or chloramphenicol and

confirmed by PCR. Each gene was expressed from the arabinose-inducible and glucose-repressible pBAD33 promoter (P_{BAD}). Repression from the *araC* promoter was achieved by growth in medium containing 0.2% (w/v) *D*-glucose and induction was obtained by adding *L*-arabinose to a final concentration of 0.2% (w/v) [31]. Plasmid pGEMT-Gne was transferred into the *E. coli wecA* mutants by electroporation. Transformants were selected on plates containing ampicillin and confirmed by PCR.

• LPS isolation & electrophoresis

Cells were grown in LB or TSB, washed with water and dehydrated by sequential washing with methanol:chloroform (1:1) × 3, ethanol, acetone × 2 and diethyl ether. After evaporation of the last dissolvent, the LPS was extracted from the dehydrated cells. For strains producing LPS without O-antigen chains, the phenol/chloroform/light petroleum ether method [32] was used, while the phenol/water procedure [33] was used for strains producing a complete LPS (with O-antigen domain). For screening purposes, LPS was obtained from whole cells treated with proteinase K [34]. LPS samples were separated by SDS-PAGE or N-(2-hydroxy-1, 1-bis [hydroxymethyl] ethyl) glycine (Tricine)-SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and visualized by silver staining [35].

• Preparation of oligosaccharides

The LPS preparations (20 mg) were hydrolyzed in 1% acetic acid (100°C, 120 min) and centrifuged (8000 × *g*, 30 min). To obtain the lipid A, the precipitates were collected and lyophilized. The oligosaccharide fractions: high-molecular-mass polysaccharide (PS), LPS core-oligosaccharide (OS) and sometimes intermediate fractions were obtained by fractionation of the supernatants. Fractions of supernatants were obtained on a column (56 × 2.6 cm) of Sephadex G-50 (S) in 0.05 M pyridinium acetate buffer, pH 4.5 and monitored by a differential refractometer.

• Gas chromatography–mass spectrometry analysis

The PS fraction was hydrolyzed with two M CF_3CO_2H for 2 h at 100°C, and the monosaccharides were conventionally converted into methylated alditol acetates and methyl glycoside acetates. The converted sugars were analyzed on an Agilent Technologies 5973N MS instrument equipped with a 6850A GC and an RTX-5 capillary column (Restek, 30 m × 0.25 mm id, flow rate 1 ml/min, He as carrier gas). Analysis of acetylated methyl glycosides was performed with the following temperature program: 150 for 5 min, 150→250 at 3°C/min, 250 for 10 min. The program used for partially methylated alditol acetates was: 90°C for 1 min, 90°C→140°C at 25°C/min, 140°C→200°C at 5°C/min, 200°C→280°C at 10°C/min, 280°C for 10 min.

• Isolation & purification of enterobacterial common antigen

The procedure was employed as previously described [36]. Briefly, bacterial cells were extracted by a combined hot phenol-water extraction (9-48) and phenol-chloroform-petroleum (PCP) ether fractionation (8-11). After dialysis and lyophilization, the aqueous phase of the phenol-water extraction was treated with the PCP mixture. The LPS precipitated when some drops of water were added to the phenol phase, while enterobacterial common antigen (ECA) remained in solution and was subsequently recovered by extensive dialysis and lyophilization. This fraction was resuspended in water and centrifuged at 105,000 × *g* for 4 h. The lyophilized supernatant is enriched in ECA and was further purified by column chromatography on diethylaminoethyl-cellulose with stepwise elution with 0.5, 1.0 and 1.6 M ammonium acetate-methanol buffer [37]. ECA is eluted in the middle fraction and, after dialysis was lyophilized. For quantitative determination of mannosaminuronic acid, the ECA fraction was reduced with NaBH4 after reaction with the carbodiimide reagent and hydrolysis with 4 M HC1 for 6h. Sugars (GlcN and ManN) in the reduced polymer were found to be substrates for enzymatic phosphorylation by hexokinase/ATP [38].

• Deoxycholate sensitivity assay

Five-microliter portions of the appropriate dilutions of exponential cultures of the wild-type and mutant strains were incubated for 24 h at 37°C on an LB plate or an LB plate containing 1% deoxycholate (DOC). The minimum inhibitory concentration (MIC) was calculated according to their inability to grow on LB plates containing several DOC concentrations. Similar amount of bacteria were inoculated in the original drops.

Results

Escherichia coli has so many O-antigen serogroups, but majority are Wzy-dependent and the initial sugar of the repeat subunit is *N*-acetyl-D-glucosamine

(D-GlcNAc). When D-GlcNAc is not present, *N*-acetyl-D-galactosamine (D-GalNAc) takes its place. Only in two strains (O45 and O62D1), *N*-acetyl-D-fucosamine (D-FucNAc) has been found at the reducing end [5]. To study the relation between first sugar of the O-antigen repeat unit and the Wzy polymerase, we need two different affinity systems. Given the O:34-antigen repeat unit of *A. hydrophila* AH-3 have D-GalNAc at the reducing end **(Figure 1A)**, we decided to use *E.coli* O-antigen serogroups with D-GlcNAc at the reducing end of repeat unit. The sugar variability at the nonreducing end of *E. coli* serogroups is too high; therefore, we used three serogroups with different sugars at the nonreducing-end: O7 has 4-acetamido-4, 6-dideoxy-D-glucose (D-Qui4NAc); and O150 and O159 have D-GlcNAc **(Figure 1B & D)**. Furthermore, we used the ECA, which has D-GlcNAc at the reducing end and 4-acetamido-4, 6-dideoxy-D-galactose (D-Fuc4NAc) at the nonreducing end **(Figure 1E)**. In *E. coli* O7, O150 and O159, the transfer of D-GlcNAc to Und-P is performed by WecA [5], whose gene encoding is in the ECA cluster. In *A. hydrophila* O34 the proximal sugar is D-GalNAc **(Figure 1A)**, and the transfer of this sugar to Und-P is performed by WecP [7], whose gene encoding is in the O-antigen cluster. WecA and WecP are both UDP-HexNAc:polyprenol-P HexNAc-1-P transferases.

• Cross-complementation studies in *E. coli* MV501 (*wecA* mutant from serotype O7)

The *E. coli* MV501 mutant is unable to add the initial sugar D-GlcNAc to the Und-P and therefore, is unable to biosynthesize the O7-antigen subunit (**Figure 2**, Lane 2) [18]. When plasmid pBAD33-$WecA_{Ec}$ (carrying the *E. coli* VW187 [O7] *wecA*) was introduced in *E. coli* MV501 mutant and expressed with arabinose, we could see identical LPS profile as their wild-type strain, *E. coli* VW187 serotype O7 (**Figure 2**, Lanes 5

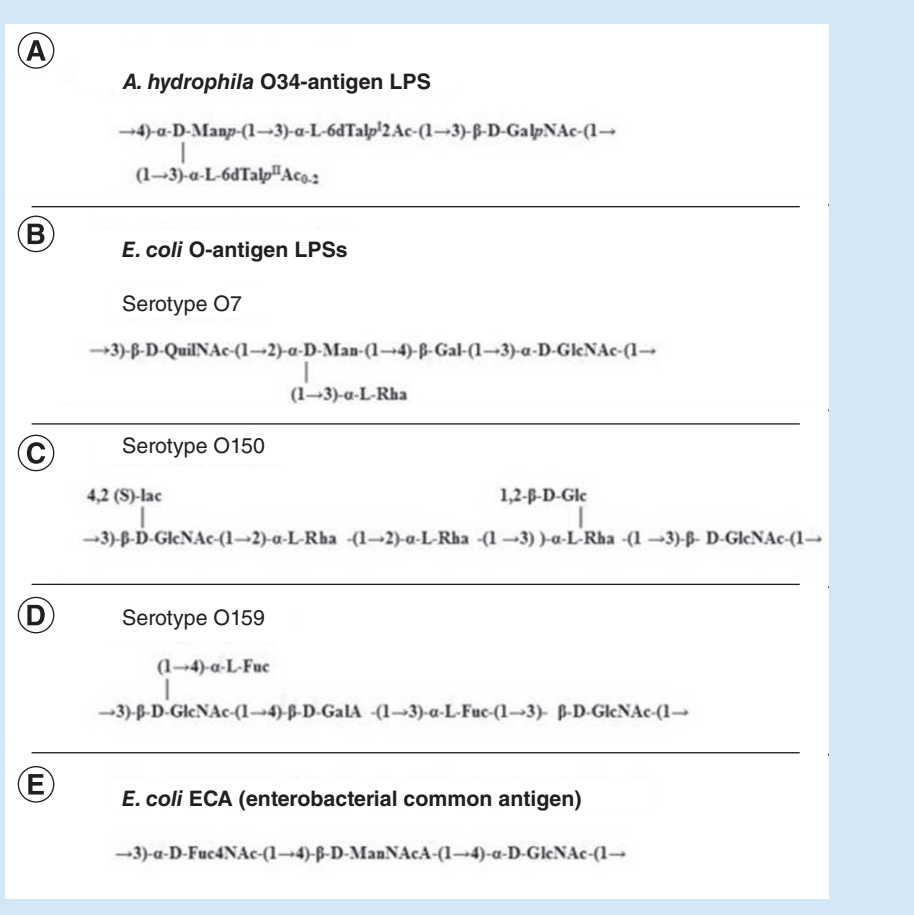
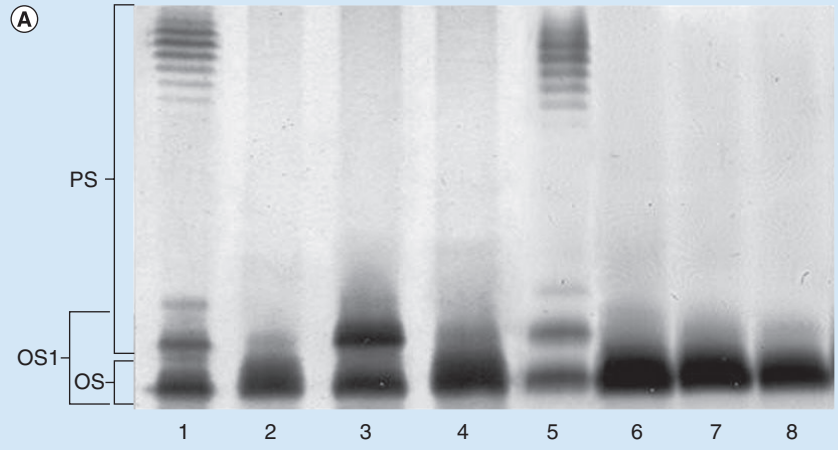


Figure 1. Chemical structures. (A) *Aeromonas* O34-antigen LPS [39], **(B)** *Escherichia coli* O-antigen LPS from serotypes O7 [40], **(C)** O150 [41] and **(D)** O159 [42] and **(E)** *E. coli* ECA [43].
ECA: Enterobacterial common antigen; LPS: Lipopolysaccharide.



Lane	Strain	Gene knocked	Gene introduced	Plasmid
1	VW187			
2	VW187	*wecA*		
3	VW187	*wecA*	*wecP*$_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
4	VW187	*wecA*	*wecP*$_{Ah}$*wzy*$_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
5	VW187	*wecA*	*wecA*$_{Ec}$ VW187	pBAD33-Gm
6	VW187	*wecA*	*wecP*$_{Ah}$ AH-3	pBAD33-Gm
7	VW187	*wecA*	*wecP wzy*$_{Ah}$ AH-3	pBAD33
8	VW187	*wecA*	*gne* AH-3	pGEMTeasy

Figure 2. Polyacrylamide gels showing the migration of lipopolysaccharide from *Escherichia coli* MV501 (wecA mutant) and its complementation. The lipopolysaccharide samples were separated on SDS-PAGE **(A)** and SDS-Tricine-PAGE **(B)** and visualized by silver staining. Shown are lipopolysaccharide samples from *E. coli* VW187 (wild-type) (Lane 1), MV501 (Lane 2), MV501 + pBAD33-WecP and pGEMT-Gne (Lane 3), MV501 + pBAD-WecP-Wzy$_{Ah}$ and pGEMT-Gne$_{Ah}$ (Lane 4), MV501 + pBAD-WecA$_{Ec}$ (Lane 5), pBAD-WecP$_{Ah}$ (Lane 6), pBAD-WecP-Wzy$_{Ah}$ (Lane 7) and MV501 + pGEMTeasy-Gne$_{Ah}$(Lane 8). Lipopolysaccharide fractions: OS, core oligosaccharide fraction; OS1, core oligosaccharide with a single repeat unit; and PS, O-antigen fraction. All the strains harboring pBAD plasmids were grown under induced conditions (+ arabinose) as indicated in the 'Materials & methods' section.

& 1). According to their LPS profiles on gels, neither the plasmid vector alone nor plasmid pBAD-WecP$_{Ah}$ (carrying the *A. hydrophila* AH-3 *wecP*) fully complemented the mutant under expressing conditions (+ arabinose) (**Figure 2**, Lane 6).

In *A. hydrophila* AH-3, WecP transfers D-GalNAc to Und-P. To obtain this initial sugar, *Aeromonas* require the activity of the Gne enzyme, which is a UDP-GalNAc4-epimerase responsible for the conversion of UDP-GlcNAc to UDP-GalNAc [22]. When plasmid pBAD33-WecP$_{Ah}$+ pGEMT-Gne$_{Ah}$ was introduced in *E. coli* MV501 mutant and expressed with arabinose, it showed an LPS profile on gels with

two bands (one with the same mobility as the mutant and the other one with a reduced mobility) (**Figure 2**, Lane 3). However, no changes could be observed when the mutant carries the plasmid pGEMT-Gne_{Ah} alone (**Figure 2**, Lane 8). LPS isolated from *E. coli* MV501 mutant with plasmids pBAD33-$WecP_{Ah}$+pGEMT-Gne_{Ah} together grown under expressing conditions (+ arabinose) showed by sugar analysis a single O-antigen repeat unit in the *E. coli* O7 background which contain D-GalNAc **(Figure 3)**.

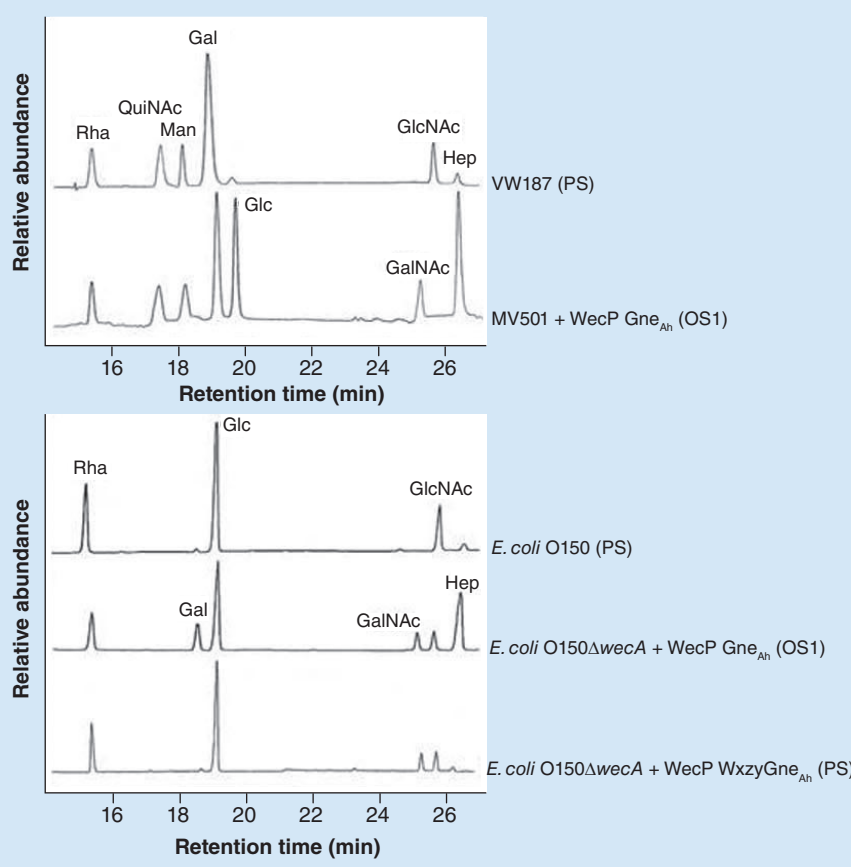
When we introduced plasmid pBAD-WecP-Wzy_{Ah} into *E. coli* MV501 mutant with or without plasmid pGEMT-Gne_{Ah}, and grown under expressing conditions (+ arabinose) both showed an LPS profile on gels with a single band with a mobility identical to the *E. coli* MV501 mutant alone (**Figure 2**, Lanes 4 & 7). It seems that the introduction of *wzy* from *A. hydrophila* AH-3 (serotype O34) causes the loss of the additional LPS band with reduced mobility observed in the same mutant strain without this gene. The sugar analysis of LPS isolated from *E. coli* MV501 mutant with plasmids pBAD-WecP-Wzy_{Ah}+pGEMT-Gne_{Ah} together, grown under expressing conditions (+ arabinose), shown the lack of monosaccharide mannose in the *E. coli* O7-antigen LPS [44], which means the loss of the single O7-antigen LPS repetition **(Figure 3)**. To evaluate the ability of Wzy_{Ah} to polymerize other *E. coli* surface polysaccharides and given that *wecA* and $wzyE_{Ec}$ belong to the ECA gene cluster [45], we analyzed the ability of complemented mutants to produce ECA. It has been described that mutants unable to produce ECA showed sensitivity to DOC [46]. We tested the MIC for DOC as it is shown in **Table 2**. The wild-type strain is resistant to DOC, while *E. coli* MV501 (*wecA* mutant) is sensitive. The introduction in the MV501 mutant of pBAD33-$WecP_{Ah}$+pGEMT-Gne_{Ah} plasmids (a single O7-repeat unit) increases slightly (twofold) their DOC resistance. However, introduction of pBAD-WecP-Wzy_{Ah}+pGEMT-Gne_{Ah} plasmids allow the practically recovering of the DOC resistance, since it is similar as the wild-type strain. No changes were observed when the plasmid vector alone was introduced.

These results prompted to us to purify core LPS-linked ECA fractions from several strains as indicated in the 'Materials & methods' section. **Table 2** shows that wild-type strain was able to synthesize ECA_{LPS} fraction (150 µg per 1 l of cell culture at OD_{600} = 1.2) detected as aminosugars after reduction and HCl hydrolysis (17.5 µg of mannosamine [ManN] and 19.8 µg of glucosamine [GlcN] per 1 l of culture). No such ECA_{LPS} fraction was obtained in *E. coli* MV501 *wecA* mutant. A large amount of ECA-like fraction could be detected when *E. coli* MV501 harbored plasmids pGEMT-Gne_{Ah} + pBAD-WecP-Wzy_{Ah} (557 µg per 1 l of cell culture at OD_{600} = 1.2) with an amount of ManN (64.0 µg) and GalN (71.3 µg) per liter of culture.

• Cross-complementation studies on *E. coli wecA* mutants from serotypes O150 & O159

The *E. coli* O7-antigen repeat unit is a pentasaccharide that contains 4-acetamido-4,6-dideoxy-D-glucose (D-Qui4NAc) at its nonreducing end **(Figure 1B)** [40], which is an uncharacteristic sugar in *E. coli* O-antigens [47]. For this reason, we tested other *E. coli* strains from serotypes not containing these deoxyamino monosaccharides **(Figure 1C & D)**.

We obtained as described in the 'Materials & methods' section in-frame *wecA* mutants O150Δ*wecA* and 0159Δ*wecA* from *E. coli* strains O25b-ST131 and CCUG 36899 (O150 and O159, respectively) [19]. These mutants were unable to produce O-antigen LPS as could be observed in **Figures 4 & 5** according to their LPS profile in gels. Both mutants showed identical LPS profile as their wild-type strain O25b-ST131 or CCUG 36899 (O150 and O159, respectively) when we introduced plasmid pBAD33-$WecA_{Ec}$. According to their LPS profiles on gels, neither the plasmid vector alone nor plasmid pBAD-$WecP_{Ah}$ or pGEMT-Gne_{Ah} alone fully complemented the mutants under expressing conditions (+ arabinose). When we introduced pBAD-$WecP_{Ah}$ and pGEMT-Gne_{Ah} together in the O150Δ*wecA* or O159Δ*wecA* mutant, as previously described for *E. coli* MV501 (WecA), their LPS profile on gels showed two bands (one with the same motility as the mutant and the other one with a reduced motility), always when cells were grown under inducing conditions (+ arabinose; **Figures 4 & 5**, Lane 3). Sugar analysis of purified LPS from these *E. coli* WecA mutants harboring pGEMT-Gne_{Ah} + pBAD33-$WecP_{Ah}$, grown under inducing conditions, showed rhamnose (Rha) in the case of O150Δ*wecA* + pGEMT-Gne_{Ah} and pBAD33-$WecP_{Ah}$ strain and fucose or galacturonic acid (Fuc and GalA, respectively) in the case of O159Δ*wecA* + pGEMT-Gne_{Ah} and pBAD33-$WecP_{Ah}$strain **(Figure 3)**. The presence of both sugars with the LPS profile in gels suggested a single *E. coli* O150-antigen or O159-antigen



B

	nmoles/mg of LPS fraction					
OS, OS1 or Ps Fraction	GlcNAc	GalNAc	Gal	Man	Rha	QuiNAc
VW187 (PS)	198	0	588	196	192	184
MV501 + WecP Gne $_{Ah}$ (OS1)	0	125	380	122	118	115
MV501 + WecP Wzy Gne $_{Ah}$ (OS)	0	0	246	0	0	0

	GlcNAc	GalNAc	Glc	Rha
E. coli O150 (PS)	374	0	740	561
E. coli O150 Δ*wecA*+ WecP Gne $_{Ah}$ (OS1)	72	85	390	248
E. coli O150 Δ*wecA* + WecP Wzy Gne $_{Ah}$ (PS)	189	194	747	568

	GlcNAc	GalNAc	Fuc	GalA
E. coli O159 (PS)	582	0	578	285
E. coli O159 Δ*wecA* +WecP Gne (OS1)	134	142	272	269
E. coli O159 Δ*wecA* + WecP Wzy Gne $_{Ah}$ (PS)	276	287	562	498

Figure 3. Gas–liquid chromatography of purified lipopolysaccharide fractions from *Escherichia coli* mutants and its complementations. (A) Gas-liquid chromatograms of purified lipopolysaccharide fractions (OS: core oligosaccharide fraction; OS1: core oligosaccharide with a single repeat unit; and PS, O-antigen fraction) from the *E. coli*Δ*wecA* mutants and its complementation. **(B)** Monosaccharide contents of these lipopolysaccharide fractions determined by GLC. The identity of the polysaccharides is as follows: Gal: Galactose; GalA: D-Galacturonic acid; GalNAc: N-D-acetyl-galactosamine; GlcNAc: N-acetyl-D-glucosamine; D-Glc: D-glucose; Fuc: D-Fucose; Hep: L-D-heptoses; Man: D-Mannose; QuiNAc: *N*-acetyl-D-quinovosamine; Rha: L-Rhamnose.

Table 2. Sensitivity to deoxicholate measured as minimum inhibitory concentration and enterobacterial common antigen production in *Escherichia coli* VW187 (serotype O7) and mutants.

Strain	DOC (MIC)	ECA production†
Wild type	1%	+ (150 μg/l)
MV501 (*wecA* mutant)	0.15%	- (<1 μg/l)
MV501 + pGEMT-Gne_{Ah} + pBAD33-$WecP_{Ah}$	0.3%	NT
MV501 + pGEMT-Gne_{Ah}	0.15%	NT
MV501 + pGEMT-Gne_{Ah} + pBAD33-WecP-Wzy_{Ah}	0.8%	+++ (557 μg/l)
MV501 + pBAD33-WecP-Wzy_{Ah}	0.15%	NT

†The strains carrying pBAD plasmids were grown under inducing conditions (+ arabinose).
+: Positive; -: Negative; +++: Highly positive. The numeric value corresponds to per 1 l of cells culture at OD_{600} = 1.2 as described in the 'Materials & methods' section.
DOC: Deoxycholate; ECA: Enterobacterial common antigen; MIC: Minimum inhibitory concentration; NT: Not tested.

LPS repeat unit [41,42]. LPS isolated from *E. coli* WecA mutants in both cases lack Rha and Fuc/GalA, two monosaccharides absent in the different *E. coli* LPS cores described [41,42]. However, when both mutants carry the following plasmids: pGEMT-Gne_{Ah} + pBAD-WecP-Wzy_{Ah}, altogether but not separately, and were grown under inducing conditions a full recovery of their specific O-antigen LPS, judged by their LPS profile in gels is achieved (**Figures 4 & 5**, Lane 4). When we purified the LPS from these *E. coli* WecA mutants harboring pGEMT-Gne_{Ah} + pBAD-WecP-Wzy_{Ah} we identified Rha in the case of O150 strain and Fuc/GalA in the case of O159 strain by sugar analyses **(Figure 3)** [41,42]. In both cases the amount of Rha or Fuc were several times higher (ratio Rha/Hep or Fuc/Hep) than previously indicated for the same mutants harboring only pGEMT-Gne_{Ah} and pBAD33-$WecP_{Ah}$ plasmids. These results, in addition to the LPS profiles in SDS gels, suggested the presence of several O-antigen repetition units when Wzy_{Ah} is added. No changes in their LPS profile were observed when both WecA mutants harbored pBAD33-WecP-Wzy_{Ah} without pGEMT-Gne_{Ah} (**Figures 4 & 5**, Lane 5).

• Topology of O-antigen polymerases (Wzy)

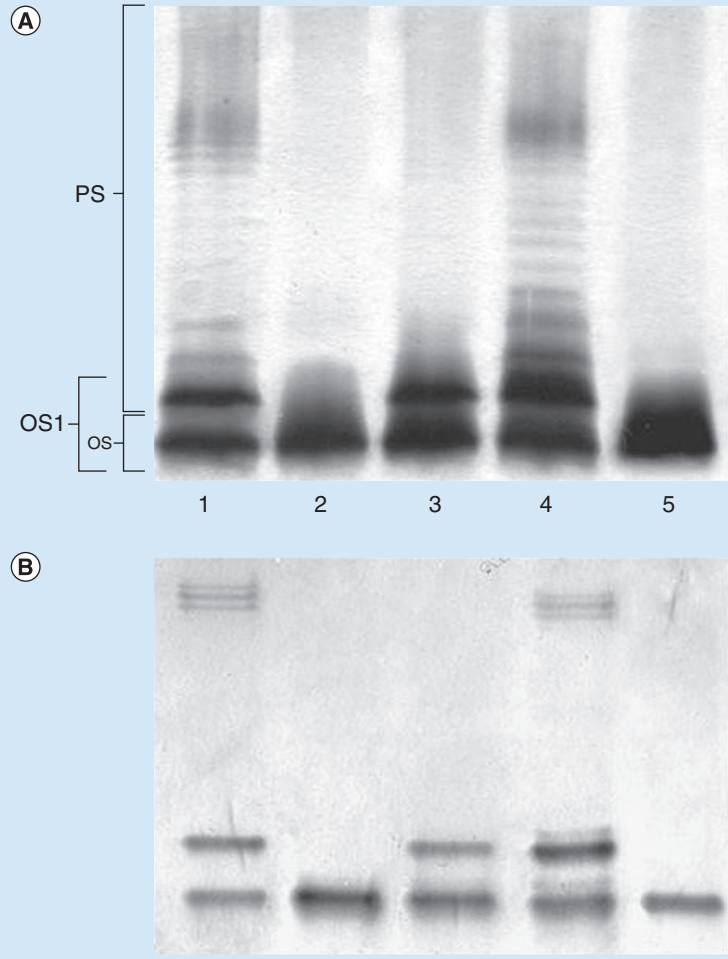
The O-antigen polymerases are generally extremely hydrophobic and have ≥11 putative transmembrane domains [48–50]. However, topology maps predicted by TMMTOP v2.0 [29] show that, except for the *E. coli* DH5α Wzy-ECA ($WzyE_{Ec}$) which contains 13 putative transmembrane domains, the remaining O-antigen polymerases of strains studied contains a lower number of transmembrane domains (TMs): ten TMs are present in *E. coli* Wzy-O150, nine TMs in *E. coli* Wzy-O159 and the *A. hydrophila* Wzy-O34 (Wzy_{Ah}) and seven TMs in *E. coli* Wzy-O7 **(Figure 6)**. Furthermore, the averages of hydropathicity (GRAVY) of the O-antigen polymerases $WzyE_{Ec}$, Wzy_{Ah} and Wzy-O159 are lower (0.81, 0.82 and 0.89, respectively) than the estimated for Wzy-O150 (1.04) and higher than the estimated for Wzy-O7 (0.66).

The *E. coli* DH5α Wzy-ECA contains six periplasmic loops (PLs), being PL2 and 5 the larger ones (each with 22 amino acid residues). The *E. coli* Wzy-O150, Wzy-O159 and the *A. hydrophila* Wzy-O34 are predicted to have four PLs, being PL1, 2 and 3 the larger ones in *E. coli* Wzy-O150 and Wzy-O159 (30, 26 and 30 amino acid residues; and 43, 26, 40 amino acid residues, respectively); and PL 3 and 4 the larger ones in *A. hydrophila* Wzy-O34 (44 and 88 amino acids). The *E. coli* Wzy-O7 only contains three PLs being PL 3 the larger with 146 amino acids **(Figure 6)**. As described in *Shigella flexneri* and *Pseudomonas aeruginosa* [49,50], *A. hydrophila* Wzy-O34 shows two RX_nG motifs in PL3 and PL4 ($RX_{15}G$ and $RX_{17}G$, respectively). However, *E. coli* Wzy-ECA and Wzy-O150 contain a motif RX_nG in PL2 ($RX_{13}G$ and $RX_{17}G$, respectively) and a motif HX_nG in PL5 and PL1 ($HX_{10}G$ and $HX_{12}G$, respectively). In contrast, *E. coli* Wzy-O159 contains three RX_nG motifs: a $RX_{15}G$ motif on PL1 and 3; and a $RX_{17}G$ in PL2. The *E. coli* Wzy-O7 contains three RX_nG motifs and all of them localized in PL3 ($RX_{10}G$, $RX_{17}G$ and $RX_{22}G$) **(Figure 6)**.

Discussion

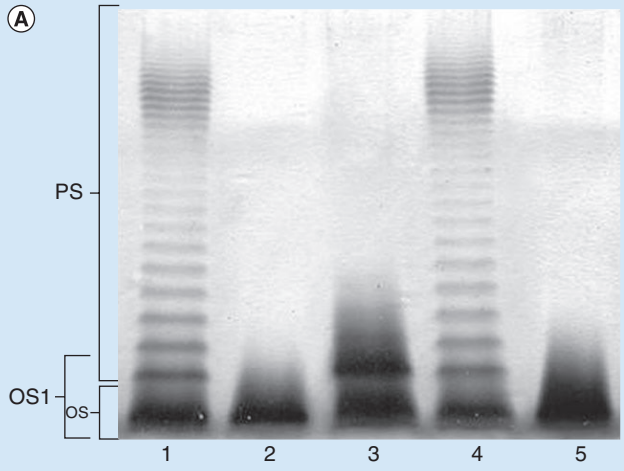
Many *E. coli* O-antigen repeat units have *N*-acetylglucosamine-phosphate (GlcNAc-1-P) as first sugar, which is transferred to the Und-P by WecA, to give Und-PP-linked GlcNAc [5]. Other *E. coli* O-antigens have *N*-acetylgalactosamine-phosphate (GalNAc-1-P) at the reducing end, and just two have *N*-acetylfucosamine-phosphate

(FucNAc-1-P) [47]. In *E. coli* O157, GalNAc-UndP is synthesized from GlcNAc-UndP by an epimerase capable of interconverting GlcNAc-P-P-Und and GalNAc-P-P-Und [51]. However, in *A. hydrophila* AH-3 the GalNAc-1-P is synthetized by the Gne epimerase, which have two enzymatic activities: UPD-Gal and UDPGalNAc 4-epimerimerase. Gne is responsible for the conversion of UDPGlcNAc to UDPGalNAc and the transfer of GalNAc-1-P to Und-P to give Und-PP-linked



Lane	Strain	Gene knocked	Gene introduced	Plasmid
1	*E. coli* O150			
2	*E. coli* O150	*wecA*		
3	*E. coli* O150	*wecA*	$wecP_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
4	*E. coli* O150	*wecA*	$wecP_{Ah}wzy_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
5	*E. coli* O150	*wecA*	$wecP_{Ah}$ wzy_{Ah} AH-3	pBAD33-Gm

Figure 4. Polyacrylamide gels showing the migration of lipopolysaccharides from *Escherichia coli* O150ΔwecA mutant and its complementation. The lipopolysaccharide (LPS) samples were separated on SDS-PAGE **(A)** and SDS-Tricine-PAGE **(B)** and visualized by silver staining. Shown are LPS samples from 025b-ST131 wild-type serotype O150 (Lane 1), O150Δ*wecA* (Lane 2), O150Δ*wecA* + pBAD33-$WecP_{Ah}$ and pGEMT-Gne (Lane 3), O150Δ*wecA* + pBAD-WecP-Wzy_{Ah} and pGEMT-Gne (Lane 4) and O150Δ*wecA* + pBAD-WecP-Wzy_{Ah} (Lane 5). LPS fractions: OS: core oligosaccharide fraction; OS1: core oligosaccharide with a single repeat unit; and PS: O-antigen fraction. All the strains harboring pBAD plasmids were grown under induced conditions (+ arabinose) as indicated in the 'Materials & methods' section.



Lane	Strain	Gene knocked	Gene introduced	Plasmid
1	*E. coli* O150			
2	*E. coli* O150	*wecA*		
3	*E. coli* O150	*wecA*	$wecP_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
4	*E. coli* O150	*wecA*	$wecP_{Ah}wzy_{Ah}$/*gne* AH-3	pBAD33-Gm/pGEMTeasy
5	*E. coli* O150	*wecA*	$wecP_{Ah}$ wzy_{Ah} AH-3	pBAD33-Gm

Figure 5. Polyacrylamide gels showing the migration of lipopolysaccharide from *Escherichia coli* O159ΔwecA mutant and its complementation. The LPS samples were separated on SDS-PAGE **(A)** and SDS-Tricine-PAGE **(B)** and visualized by silver staining. Shown are lipopolysaccharide samples from CCuG36899 wild-type serotype O159 (Lane 1), O159Δ*wecA* (Lane 2), O159Δ*wecA* + pBAD33-$WecP_{Ah}$ and pGEMT-Gne (Lane 3), O159Δ*wecA* + pBAD-WecP-Wzy_{Ah} and pGENT-Gne (Lane 4) and O159Δ*wecA* + pBAD-WecP-Wzy_{Ah} (Lane 5). LPS fractions: OS: core oligosaccharide fraction; OS1: core oligosaccharide with a single repeat unit; and PS: O-antigen fraction. All the strains harboring pBAD plasmids were grown under induced conditions (+ arabinose) as indicated in the 'Materials & methods' section.

GalNAc, is performed by the initial transferase WecP [7].

The *E. coli* O7-antigen repeat unit is a pentasaccharide of D-GlcNAc, galactose (Gal), D-mannose (Man), Rha and 4-acetamido-4,6-dideoxy-D-glucose (D-Qui4NAc) [40] **(Figure 1B)**. The abolishment of WecA, which transfers the GlcNAc-1-P to Und-P [5], leads to the absence of O7-antigen repeat unit (**Figure 2**, Lane 2). In this *E. coli* O7 background, the *A. hydrophila* WecP, which transfers the GalNAc-1-P to Und-P [7], is unable to restore the LPS profile (**Figure 2**, Lane 6). *Escherichia coli* O7 lacks Gne epimerase and, only when this gene is present and expressed this strain is able to obtain the GalNAc required for the WecP activity [22]. The *E. coli* O7 *wecA* mutant

carrying *gne* + *wecP* in expressing vectors reveals an LPS profile on gels with two bands (**Figure 2**, Lane 3), characteristic of an LPS-core with a single O-antigen repeat, and the chemical data indicate the presence of GalNAc **(Figure 3)**. These data suggest that GalNAc is the first sugar of the repeat unit and *E. coli* Wzx-O7 is able to translocate it, but *E.coli* Wzy-O7 does not have the ability to polymerize the O-antigen repeat units. Surprisingly, *E. coli* O7 *wecA* mutant with *gne* + *wecP-wzy*$_{Ah}$ loses the characteristic LPS double band and shows a single band (**Figure 2**, Lane 4) and production of ECA-like molecules. The chemical data indicate the lack of the O7-antigen single repeat when the *wecA* mutant carries *gne* + *wecP-wzy*$_{Ah}$ **(Figure 3)**. By sensitivity to DOC and chemical methods, we show that ECA-like molecules were produced when the *wecA* mutant carries *gne* + *wecP-wzy*$_{Ah}$, which were not previously found when the *wecA* mutant carried only *gne* + *wecP* **(Table 2)**. Similar results were seen in the *E. coli* O121 background whose O-antigen repeat unit is a tetrasaccharide containing GlcNAc at the reducing end and 4-(*N*-acetylglycyl) amino-4,6-dideoxy-*D*-glucose, *D*-Qui4N(*N*-acetyl-glycyl), at the nonreducing end (data not shown).

The repeat units of the *E. coli* O150 and O159-antigens are a hexasaccharide and a pentasaccharide, respectively **(Figure 1C & D)**, which contain β-D-GlcNAc at both the reducing and nonreducing ends. As in *E. coli* O7, the *wecA* mutation abolishes formation of the O-antigen repeat unit

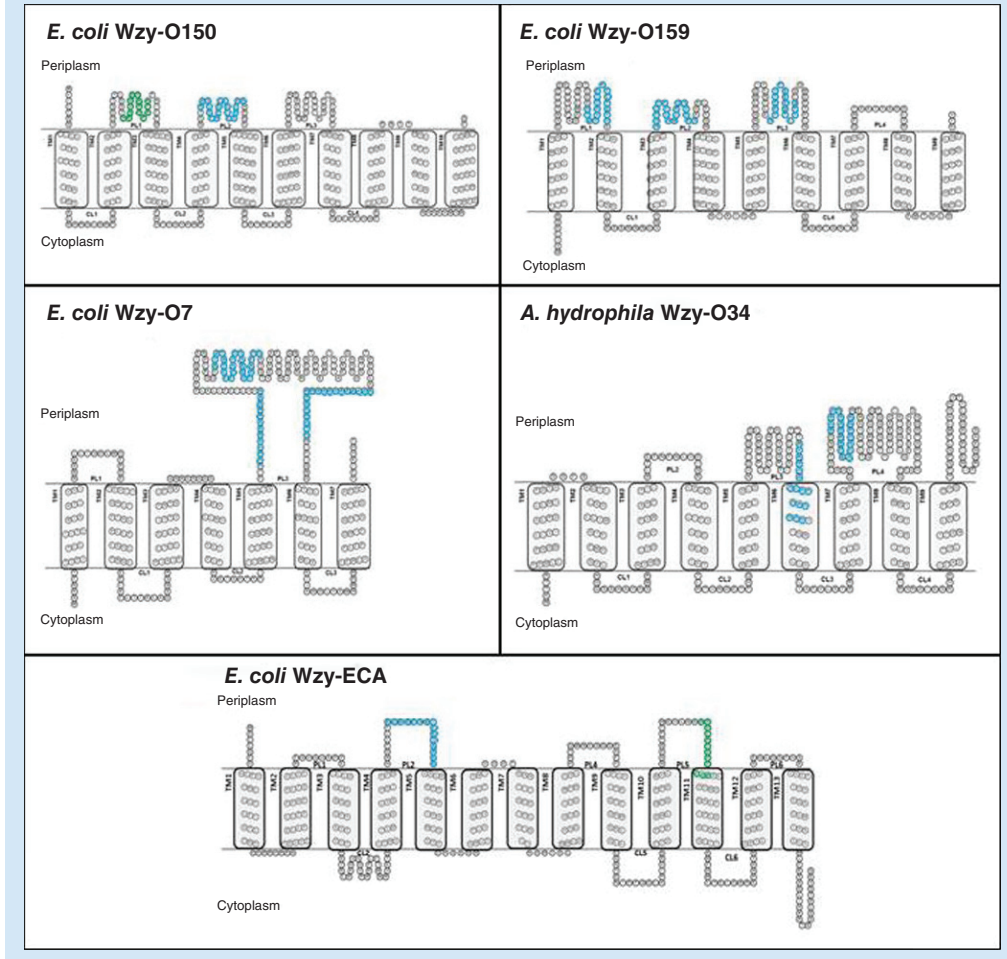


Figure 6. Topological comparison of O-antigen polymerases. Theoretical topology map of the O-antigen polymerases in *Escherichia coli* WzyE$_{Ec}$ (enterobacterial common antigen Wzy polymerase), Wzy-O150, Wzy-O159 and Wzy-O7; and *A. hydrophila* Wzy-O34 (Wzy$_{Ah}$). Topology maps were predicted by TMMTOP v2.0 [29]. PL1-n, number periplasmic loops; CL1-n, number cytoplasmic loops; and TM1-n, number transmembrane domains. Blue circles show RX$_n$G motifs and green circles HX$_n$G motifs.

(**Figures 4 & 5**, Lane 2) and *A. hydrophila* WecP is unable to perform cross-complementation. However, when *A. hydrophila gne* + *wecP* were introduced together in the *E. coli wecA* mutants, a characteristic LPS profile with two bands was observed (**Figures 4 & 5**, Lane 3), and the chemical data indicated the presence of GalNAc in the O-antigen repeat unit **(Figure 3)**. In contrast to what was observed in *E. coli* O7, the concerted action of *A. hydrophila* Wzy_{Ah}, with the corresponding WecP in the presence of Gne is able to polymerize the corresponding O150 or O159-antigen units (**Figures 4 & 5**, Lane 4). The chemical data obtained allow us to indicate that the O-antigen LPS correspond to *E. coli* serotypes O150 or O159-like **(Figure 3)**. Therefore, in *E. coli* O150 or O159 backgrounds, the results are similar to the ones obtained in *A. hydrophila* O34 [24], since the AH-3Δ *wecP* mutant with the *E. coli* WecA, from the ECA cluster, is able to elongate the chain with a single O-antigen unit, but polymerization of the O34-antigen is only produced when *E. coli* WecA-$WzyE_{Ec}$ (both from the ECA cluster) are present together. Furthermore, plasmid pBAD-$WzyE_{Ec}$[carrying the *E. coli* ECA-Wzy] is unable to restore the Wzy_{Ah} function and polymerize the O:34-antigen when was introduced in the *A. hydrophila* AH-3 Wzy_{Ah} mutant (A405-wzy) and expressed with arabinose.

Figure 1 shows the chemical structure of the *A. hydrophila* O34-antigen LPS, *E. coli* O7, O150, O159-antigen LPSs and ECA from *E. coli*. For all these O-antigen polysaccharides the initial sugar linked to the Und-P is a HexNAc. The *E. coli* O7-antigen LPS repeating units, contain at the distal position (nonreducing end) 4-acetamido-4,6-dideoxy-*D*-glucose (*D*-Qui4NAc), which is an unusual sugar for prokaryote polysaccharides. We suggest that *A. hydrophila* Wzy is unable to recognize D-Qui4NAc at the nonreducing end of the O7 repeating unit to form a glycosidic bond with the reducing end of the growing chain. However, it is able to recognize 4-acetamido-4, 6-dideoxy-D-galactose (D-Fuc4NAc) and produces ECA in the *E. coli* O7 background **(Figure 1E)**, as shown the DOC resistance and the ECA amount in the *E.coli* MV501 with pBAD-WecP-Wzy_{Ah}+pGEMT-Gne_{Ah} plasmids **(Table 2)**. Nevertheless, it seems that O-antigen LPS is always the biosynthetic preference among the different Gram-negative bacterial surface polysaccharides in this background.

The O-antigen polymerases show high level of amino acid sequence diversity and absence of conserved regions, which has related to differences in the composition and structure of translocated O-antigen repeat units. Although Wzy mediates the formation of a glycosidic linkage between the nonreducing end of the new repeat subunit and the reducing end of the growing chain, it displays no homology to other known glycosyltransferases. The O-antigen polymerases are integral membrane proteins with 11–13 transmembrane domains, responsible for the polymerization of O-antigens, capsules and other cell surface polysaccharides [52,53]. However, topology maps predicted by TMMTOP v2.0 [29] show that *E. coli* Wzy-O7, Wzy-O150 and Wzy-O159, as well as *A. hydrophila* Wzy-O34 have lower number of transmembrane domains (TMs) **(Figure 6)**. The *E. coli* Wzy-O7 contains the lower number of TMs and average of hydropathicity. Two larger periplasmic loops essential for the catalytic activity of Wzy have been described in *Pseudomonas aeruginosa, Shigella flexneri* and *Francisella tularensis* (PL3 and 5) [48–50]. We also found two larger periplasmic loops in the *E. coli* Wzy-ECA and *A. hydrophila* Wzy-O34 (PL 2 and 5; and PL3 and 4, respectively). However, the *E. coli* Wzy-O150 and Wzy-O159 contain three large periplasmic loops, and *E. coli* Wzy-O7 only contains one **(Figure 6)**. It has been hypothesized that the periplasmic domain of the O-antigen polymerase is involved in bonding the O-antigen repeat units and polymerizing them into the O-antigen chain [54]. In *S. flexneri* and *P. aeruginosa* Wzy, the two larger periplasmic loops contain RX_nG motifs involved in their polymerase activity ($RX_{15}G$ and $RX_{10}G$, respectively) [49,50]. *A. hydrophila* Wzy-O34 also contains RX_nG motifs in PL3 and PL4, although with different lengths ($RX_{15}G$ and $RX_{17}G$, respectively). However, *E. coli* Wzy-ECA and Wzy-O150 contain a motif RX_nG and a motif HX_nG: $RX_{13}G$ and $HX_{10}G$ in Wzy-ECA and $HX_{12}G$ and $RX_{17}G$ in Wzy-O150. *Escherichia coli* Wzy-O159 shows three RX_nG motifs ($RX_{15}G$ in PL1 and 3, and $RX_{17}G$ in PL2), as well as *E. coli* Wzy-O7, although all of them are localized in PL3 ($RX_{10}G$, $RX_{17}G$ and $RX_{22}G$) **(Figure 6)**. All these data indicate some diversity among the different strains in Wzy polymerases, being *E. coli* Wzy-O7 by large the most different one.

Previous work demonstrated that Wzx proteins from O-antigen systems that use GlcNAc or GalNAc as initial sugar of the repeat unit were able to translocate O-antigen repeat units whose initial sugar was any of them [55], as well as *Salmonella* Wzy is able to discriminate between

O-antigen units possessing identical sugars but different internal sugar linkage [56]. However, we demonstrate that *E. coli* $WzyE_{Ec}$ (ECA Wzy polymerase) is able to polymerize the *A. hydrophila* O34 antigen although requiring the concerted action of WecA [24]. Therefore, the Wzx/Wzy O-antigen export pathway is not strain dependent, as it was suggested in some cases, it depends on the UDP-HexNAc:polyprenol-P HexNAc-1-P transferase (WecA/P) and their compatibility with the corresponding Wzy (O-antigen polymerase). Given the WecA mutation abolishes the transfer of D-GlcNAc to the Und-P and do not modify the terminal sugar of the repeat unit, the introduction of *A. hydrophila* Gne + WecP only changes the sugar at the reducing end and allows translocation of the repeat unit. However, the *E. coli* Wzy polymerase is unable to polymerize the repeat unit. We suggested that, in the Wzx/Wzy-dependent O-antigen export and assembly pathway, the activity of the O-antigen polymerase (Wzy) is dependent on the first sugar of the O-antigen repeat unit to produce the O-antigen polymerization. Furthermore, it needs the concerted action of the corresponding enzyme transferring the initial HexNAc to undecaprenol phosphate (UDP-HexNAc: polyprenol-P HexNAc-1-P transferase) to transfer the growing units from the Und-P to the nonreducing end of the new O-subunit. Our results support that some Wzy are permissive with the sugar at the nonreducing end of the O-antigen repeat unit, as observed in *Aeromonas hydrophila* Wzy-O34.

Conclusion

The Wzx/Wzy-dependent O-antigen export and assembly pathway is not as highly specific as it has been suggested previously, since the ability to polymerize the O-antigen depends on the compatibility between the UDP-HexNAc:polyprenol-P HexNAc-1-P transferase and the O-antigen polymerase (Wzy). Therefore, the system is able to assemble O-antigen units possessing different monosaccharide chemical structures when provided on a compatible first monosaccharide. Furthermore, some O-antigen polymerases are also permissive with the sugar at the nonreducing end of the O-antigen repeat unit.

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EXECUTIVE SUMMARY

- The *Aeromonas hydrophila* WecP, which transfers the GalNAc-1-P to Und-P, is unable to restore the lipopolysaccharide profile of *Escherichia coli* O7, O150 and O159 in an *E. coli wecA* background.
- *E. coli* O7, O150 and O159 *wecA* mutants show an LPS-core with a single O-antigen repetition when *A. hydrophila* WecP + Gne, a UDP-GalNAc 4-epimerase enzyme responsible for the conversion of UDP-GlcNAc to UDP-GalNAc, were transferred.
- The O-antigen polymerases (Wzy) from *E.coli* O7, O150 and O159 are unable to polymerize O-antigen repeat units in which the first sugar is GalNAc.
- *E. coli* O7 *wecA* mutant with *A. hydrophila* WecP + Gne + Wzy loses the single O-antigen repetition and produces ECA-like molecules.
- *E. coli* O150 and O159 *wecA* mutants with *A. hydrophila* WecP + Gne + Wzy are able to polymerize the corresponding O150 or O159-antigen units.
- Analyses of O-antigen polymerases show some diversity among the different strains, being *E. coli* Wzy-O7 the most different one.

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