Chapter 3

Mechanisms of O-Antigen Structural Variation of Bacterial Lipopolysaccharide (LPS)

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Additional information is available at the end of the chapter

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1. Introduction

One of the most complex topics within bacterial anatomy and physiology is that of exopolysaccharides. These molecules have various structures and functions and also provide different types of advantages to their producing microorganisms, including surface variability, resistance to innate and acquired immunity mechanisms, the ability to adhere to different surface and cell types and resistance to antibiotic activity.

These bacterial systems are closely linked to the different genera and species that contain them. The organisation and expression of the genes that code for these external structures, genetic expression mechanisms and biosynthesis routes are extremely heterogeneous.

Although numerous classic microbial physiology and biochemical studies have focused on analysis of the external structures of microorganisms, not until recently has the study of exopolysaccharides become important, due to the role of exopolysaccharides in bacterial pathogenicity and the ecology of microbial populations and their possible role in the colonisation, residence and adaptation mechanisms in various ecosystems. Among the most important exopolysaccharides are those described below.

2. The glycocalyx

Although it can not be considered a bacterial structure, the glycocalyx is a heterogeneous set of exopolymers that have diverse biochemical compositions [1]. The exopolymers are located immediately adjacent to the microorganism wall and are present as hydrophobic gels that are weakly associated with the external bacterial structures.

The production and presence of these exopolymers provide bacteria with a high degree of surface hydrophobicity that enables interaction between cellular and inert surfaces and subsequent bacterial colonisation through the development of microcolonies and biofilms.

Furthermore, the presence of certain biopolymers is related to the ability to resist antibiotic action by capturing these compounds through periplasmic glucans. An example of this is resistance to tobramycin, which is captured by cyclic-β (1,3)-glucan [2].

3. Capsules

In contrast to the glycocalyx, a bacterial capsule is a well-defined external structure with a characteristic composition for each bacterial genus and species that provides a number of advantages, which are primarily related virulence, to the producing microorganism.

One of the classic examples of the importance of the capsule is provided by *Streptococcus pneumoniae*, which is a gram-positive diplococci agent that causes pneumonia, meningitis and septicaemia. The virulent strains of this species produce a capsule that inhibits opsonisation and phagocytosis. The composition of the capsule is dependent on the producing bacteria. In the case of *Escherichia coli* K1, the capsule is formed by polysialic acid, in *S. pyogenes,* by hyaluronic acid and in *Streptococcus* group B by sialic acid. The biochemical composition of the capsules may be extremely diverse, which gives rise to a great amount of antigenic variability and presents a problem for the immunological mechanisms of the host in recognising these organisms [2].

It has been documented that the capsule participates in bacterial adhesion mechanisms and that its synthesis is stimulates by low stress conditions, such as the presence of serum, low Fe^{++} concentration and high CO_2 tensions. Although in certain microorganisms, the presence of a capsule is discreet, in others, such as *Cryptococcus neoformans*, the capsule plays an essential role in the mechanisms for aggression against the host.

4. Biofilms

Because of the production and exportation of bacterial exopolymers, the strains increase their degree of surface hydrophobicity, which facilitates interaction, adsorption and residence on a wide range of surfaces that, in principle, hinder bacterial colonisation. Regarding the production of biofilms, it has been documented that each bacterial genus and species responds to different signals from the environment and the host, as is the case of induction by tobramycin and the response capacity of the *quorum sensing* mechanisms. One of the most studied examples of the formation of bacterial biofilms is the case of *Pseudomonas aeruginosa*, which is an opportunistic pathogen that is associated with infections in immunocompromised hosts, such as cystic fibrosis (CF) patients. In CF patients colonised by *P. aeruginosa*, the bacteria exhibits two colonial phenotypes. The first phenotype is associated with the production of alginate (a polymer of mannuronic acid and glucuronic acid that forms a viscous gel around the bacteria), and the second phenotype is rough and is related to a lack of alginate. The production of alginate is a marker of virulence in which the producing strains are more aggressive than the non-producing strains. Additionally, alginate provides bacteria with the ability to form microcolonies and biofilms [3].

In the relationship between *P. aeruginosa* and the infected patient, the host exerts different types of selective pressure that favour the persistence of the mucoid strains with elevated alginate production. This biosynthesis is regulated by different bacterial signalling systems (quorum sensing) that detect changes in the host environment and modulate the metabolism to adapt. To date, it is not known precisely which signals in hosts with CF favour and allow for a predominance of mucoid strains compared to non-mucoid strains. *In vitro* studies, it has been demonstrated that both bacterial phenotypes exhibit similar behaviour and that successive passages in culture media inhibit the expression of the non-mucoid phenotype [4,5].

The physiology of bacteria that are found in biofilms is heterogeneous and depends on the specific site that the microorganism occupies in the microcolony. Nutrient gradients occur from the surface of the biofilm to the most internal parts, thereby influencing the bacterial physiology and consequently modifying the speed of growth, the generation time, the susceptibility to antibiotics (due to factors such as the presence of a diffusion barrier to antibiotics), the antigenic variability of individuals, the susceptibility to opsonisation and phagocytosis and even the alginate functions as a negative immunomodulator for the host [4].

Another example of the formation of biofilms is that produced by bacteria of the genus *Staphylococcus*, predominantly the coagulase-negative forms in which the production and excretion of biopolymers is capable of increasing cell surface hydrophobicity and, through hydrophobic interactions, adhering to surfaces. These mechanisms enable the bacteria to enter hosts, who subsequently require invasive procedures or therapeutic procedures, such as catheters. In the case of the bacterial populations that colonise the oral cavity, heterogeneous populations participate in the biofilms, of which *Streptococcus mutans* has a fundamental role in adherence because of its capacity to produce biofilms, thereby providing a high degree of surface hydrophobicity. This hydrophobicity allows the bacteria to adhere to dental enamel and begin the process of colonisation and the subsequent adherence to an increasingly heterogeneous bacterial population, which may ultimately cause harm and generate a cariogenic process.

5. Gram-negative lipopolysaccharide (LPS): Structure and function

LPS is essential in the structure and function of the external membrane of gram-negative bacterial cell walls. LPS intervenes in the transportation of hydrophobic molecules to the interior of bacterial cells and are an essential factor in host-microorganism interactions.

LPS is an amphipathic glycoconjugate that constitutes 10% to 15% of the total molecules in the external membrane and represents 75% of the total of bacterial surface [1]. There are three different LPS domains: a) Lipid A, which is the domain that is anchored to the membrane and the hydrophobic and endotoxic portions of the structure; b) The core oligosaccharide, which is the domain that connects lipid A to antigen O and is divided into the inner core and the outer core. The inner core is joined to lipid A and consists of unusual monosaccharides, including 2-keto-3-deoxy-octanoate (Kdo) and L-glycero-D-mannoheptose.

The outer core is joined to the O antigen and is made of common sugars such as hexoses and hexosamines [2]; and c) The O polysaccharide, which is the hydrophilic and immunodominant domain of LPS and is an oligosaccharide of repeated units that is projected from the core toward the exterior of the bacterial surface.

The O antigen has a polysaccharide chain that varies in length with up to 40 repeated units of dideoxyhexoses. At least 20 different sugar molecules may compose the O antigen, including molecules that are rarely found in nature, such as abequose, colitose, paratose and tyvelose. These components are strain-specific. The O antigen displays a large degree of inter-species and intra-species variation, which is related to the nature, order and union of the different sugars (**Figure 1**) [3].

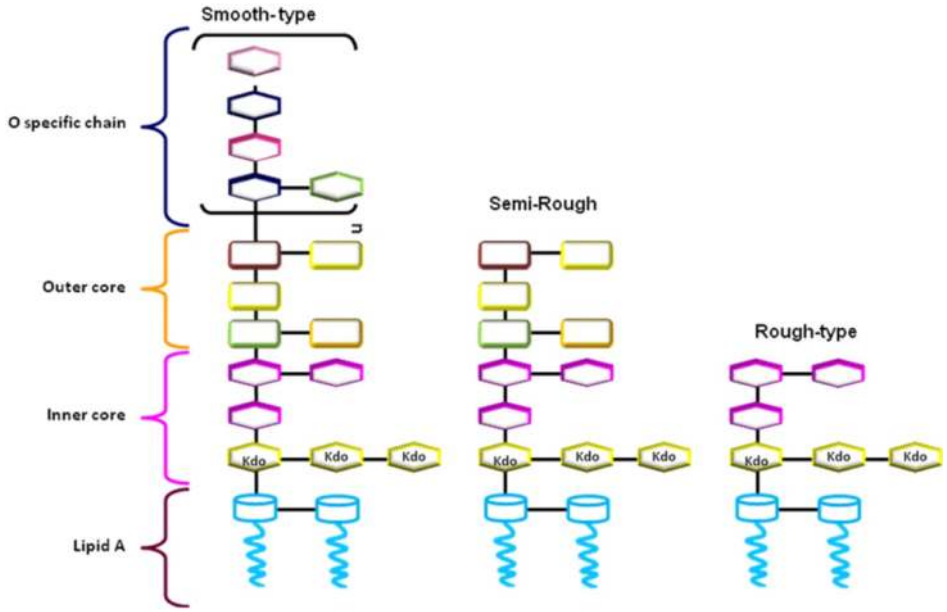


Figure 1. Schematic representation of lipopolysaccharide structure. Smooth-type (left), Semi-Rough type with only one O-chain subunit (center) and rough-type (right).

The O antigen is the immunodominant part of LPS and therefore is the easiest target for the humoral response of the host. For this reason, the O antigen is the basis for the serological classification of gram-negative bacteria. The O antigen is recognised by the innate immune response and participates in complement activation and in the inhibition of the formulation of the complex that attacks the membrane [6,7].

6. Biosynthesis of LPS

LPS is the primary component in the surface of gram-negative bacteria. The synthesis of LPS structures, which consist of lipid A, the core and antigen O, begins in the cytoplasm, where

these structures are assembled. The structures are translocated to compartments such as the periplasm until the final destination is reached, which is the surface of the external membrane. The synthesis process has been widely studied in *E. coli* and *Salmonella.* The biosynthesis and exportation pathways of LPS are common among the majority of gram-negative bacteria, as is explained below (**Figure 2**). However, unique characteristics may exist in certain bacteria with respect to the types of enzymes and particular pathways.

The formation of lipid A is carried out in the internal face of the cytoplasmic membrane, and nine enzymes participate: LpxA, LpxC, LpxD, LpxH, LpxB, LpxK, KtdA, LpxL and LpxM. The biosynthesis of LPS begins with the formation of uridine diphosphate-diacyl- *D*-glucosamine (UDP-diacyl-GlcN) from uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc). This reaction is catalysed by the enzymes LpxA, LpxC and LpxD and results in two 3-OH fatty acid chains in the 2 and 3 position of UDP-glucosamine (UDP-GlcNAc) to form UDP-2,3-diacyl- glucosamine (UDP-diacyl-GlcN). Subsequently, this molecule is hydrolysed by LpxH to form lipid X, the enzyme LpxB condenses the lipid X and its precursor UDP-diacyl-GlcN to form disaccharide-1-P and the enzyme LpxK phosphorylates this molecule at the 4 position of the disaccharide-1-P molecule to form the lipid IV_A. Subsequently, KdtA incorporates the waste from 3-deoxy-D-manno-octulosonic acid (Kdo) in the 6′ position of lipid IV_A using the nucleotide cytidine monophosphate-3-deoxy-D-manno-octulosonic acid (CMP-Kdo) as a donor to produce Kdo_2 – lipid IV_A, which is exposed to more reactions catalysed by LpxL and LpxM to form Kdo_2 – lipid A. The enzyme LpxL adds a second lauryl, and LpxM adds a residue of myristoyl to the distal glucosamine unit.

Notably, the acyltransferases – LpxA, LpxD, LpxL and LpxM – selectively catalyse the different substrates and employ different acyl donors. For the first steps of the synthesis pathway of lipid A, the enzymes LpxA, LpxB and LpxD are required, with 3R-hydroxyacyl - Acyl Carrier Protein (3R-hydroxyacyl-ACP) serving as a donor. This compound is dehydrated by FabZ to form trans-2-acyl-ACP, which is also used as a donor of fatty acids in the biosynthesis of phospholipids. The synthesis of other LPSs in bacteria, such as *Neisseria meningitidis*, also involve trans-2-acyl-ACP[8]. Importantly, the structure of lipid A is the most conserved compared to the structure of the core oligosaccharides and antigen O [9,10].

6.1. The core oligosaccharides

The assembly of lipid A from the core oligosaccharides (Kdo_2 – lipid A) is the next step in the synthesis of LPS. This step is performed on the cytoplasmic surface of the internal membrane by glycosyltransferases, which are associated with the membrane and with nucleotide sugars as donors.

The core oligosaccharides normally contains 10 to 15 monosaccharides and may be divided into two structural regions, which are the inner core and the outer core, which are ultimately connected to lipid A and antigen O, respectively, in the final structure of LPS. The inner core contains residues of Kdo and Hep (L-glycero-D-manno-heptose). Kdo is the most conserved component in the nuclear region of the LPS. In contrast, the outer core is more variable,

depend on the strain. However, the vertebral column of the oligosaccharide is typically composed of six units, and upon joining with other units, the column forms structures. The sugars commonly found in the core oligosaccharides are D-glucose, D-galactose, Kdo and Hep [9,10].

6.2. The O antigen

The majority of O antigens are heteropolymers, although a portion of O antigens may be composed of a single monosaccharide. The synthesis of the O antigen is performed in the same location as the core oligosaccharides, and this synthesis also uses nucleotide sugars as donors. In the majority of bacteria, a cluster of genes known as *rfb* codes the enzymes necessary for 1) the biosynthesis of the nucleotide sugars of antigen O, 2) the transfer of the sugars to form the polysaccharide chain (glycosyltransferases) and 3) the assembly and transfer of antigen O toward the periplasm. The synthesis routes of the nucleotide sugars are grouped according to the nucleotide that bonds to the sugar, which may be CDP, UDP, dTDP or GDP. Antigen O may be a homopolymer or a heteropolymers, and the sugars may be formed linearly or in a ramification. Glycosyltransferases may be grouped according to their function, and they carry undecaprenyl phosphate, which is also used for the synthesis of capsular polysaccharides and peptidoglycans.

The following hypotheses have been proposed regarding the assembly and transfer process of antigen O: a) a pathway dependent on Wzy, which is the prototype system; b) a pathway dependent on ABC transporters, which are typically used by linear polysaccharide structures; c) a pathway dependent on synthase, which involves glycosyltransferases capable of synthesising within a single polypeptide and is an uncommon pathway and finally d) seroconversion reactions, in which the addition of acetyl residues or glucose residues modifies antigen O. Within the prototype pathway dependent on Wzy, in bacteria such as *Salmonella enterica* and *E. coli*, a multi-step process occurs. When the lipid A-core and the O antigen are synthesised, they are transported to the periplasm. The protein MsbA, which displays homology with MDR (multi-drug resistant) eukaryote proteins, transports the lipid A-core, and Wzx transports the O antigen, which was previously polymerised by the proteins Wzy and Wzz. With the help of WaaL, the structures of the lipid A-core and the O antigen are assembled, finally producing the LPS [10].

6.3. LPS and its transportation to the external membrane

When an LPS is formed, it must pass through the periplasmic space to reach the external membrane [9,10], and this process is facilitated by protein LptA (periplasmic), LptB (cytosolic), LptC, LptF, LptG (internal membrane) and LptD and LptE (external membrane). Several of these proteins act in complexes. For example, in the case of the transporter ABC, LptBFG and LptA and LptC translocate the LPS to the internal side of the external membrane such that the proteins LptD and LptE place it on the surface of the membrane. It has been observed an absence of LptA or LptB or both causes the accumulation of LPS in the periplasm [11–17].

In the majority of bacteria, the genes that code for the enzymes involved in the biosynthesis of the O antigen are found in clusters. However, in the case of *Helicobacter pylori*, which is a pathogenic bacteria of the human stomach, these enzymes are found distributed throughout its chromosome, which probably contributes to the fact that the assembly pathway of its LPS has not been completely characterised. However, several enzymes that participate in the synthesis of *H. pylori* LPS has been identified and characterised, including several glycosyltransferases [18,19]. The glycosyltransferase WecA and the ligase WaaL also participate in the biosynthesis of *H. pylori* LPS. However, translocases are typically not involved in the translocation of the O antigen to the periplasm for its assembly with the lipid A-core structure [20]. Only the participation of a translocase named Wzk, which directs N-glycosylation in other bacteria, has been observed, and this fact suggests that the translocase Wzk of *H. pylori* could indicate an evolutionary connection between the biosynthesis pathways of LPS and glycoproteins [18]. Recently, it has been observed that there is an analogy and homology between the biosynthesis of LPS and the biosynthesis of glycoproteins in other bacteria, such as *S. enterica, P. aeruginosa* PAO1, *Neisseria* spp., *Paenibacillus alvei, Campylobacter jejuni and E. coli O8.* This homology could have enormous biotechnological potential. However, further studies are required to confirm this fact [21].

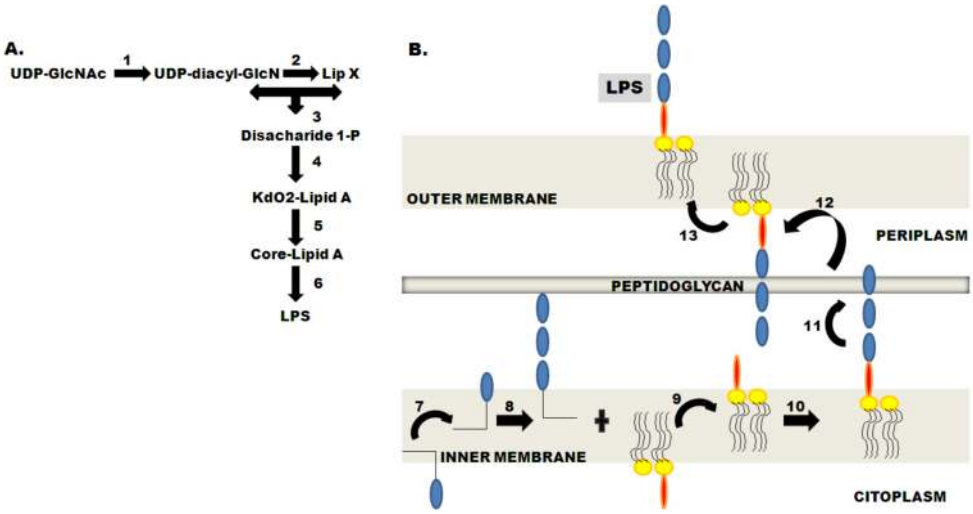


Figure 2. The biosynthetic pathway and transport of lipopolysaccharide. **A.** A representation of the biosynthetic pathway of the structures of LPS in *E. coli*. **B.** The assembly and transport of the LPS; the antigen O is assembled to the structure core-lipid A in the periplasm and is later transported toward the outer membrane. The names of the enzymes involved in these processes are: 1. LpxA, LpxC, LpxD; 2. LpxH; 3. LpxB; 4. LpxK, KdtA, LpxL, LpxM; 5. Glycosyltransferases; 6 and 10. WaaL; 7. Wzx; 8. Wzy, Wzz; 9. MsbA; 11. Lpt B C F G; 12. LptA; 13. Lpt D E. The lipid A (Yellow circles), the core (Red ovals) and antigen O (Blue ovals)

7. Regulation of the expression of LPS

The biosynthesis of LPS is performed through two separate pathways. One pathway involves the formation of lipid A and the core, and the other pathway involves the formation of the O antigen. In the synthesis of LPS, a large number of genes participate, many of which are part of clusters located in different regions of the bacterial chromosome and, in some organisms, in plasmids [22,23].

7.1. Regulation of the biosynthesis of lipid A

Lipid A and the core oligosaccharide are formed in a continuous process, which is separate from the synthesis of the O antigen. In the majority of *Enterobacteriaceae*, the genes involved in this synthesis are found in a single copy and share several characteristics with pathogenicity islands (PAI) [10,24].

The genes involved in the first steps of the biosynthesis of lipid A in *E. coli, S. enterica* serovar Typhimurium, *Yersiia enterocolitica*, *Haemophilus influenzae* and *Rickettsia rickettsii* are grouped into the *lpx*D-*fab*Z-*lpx*A-*lpx*B cluster[8]. The genes *lpx*A and *lpx*D code for N-acyltransferases, which add fatty acids to the glucosamine disaccharide. Both enzymes contain a conserved repeated structure, which is the hexapeptide $[(I,V,L)GXXXX]_n$. The gene *lpx*B is a co-transcript with *lpx*A and codes for the disaccharide synthase of lipid A, which catalyses the formation of the disaccharide of lipid A from UDP-2.3-diacylglucosamine and 2,3-diacylglucosamine-1-phosphate. The gene *fab*Z codes the enzyme that catalyses the dehydration of (3R)-hydroxyacyl-ACP to trans-2-acyl-ACP, which is used as a donor of fatty acid in the biosynthesis of phospholipids [10].

The proteins involved in the biosynthetic pathways of UDP-GlcNAc, UDP-Glc and UDP-Gal are coded in constitutive genes.

L-glycero-D-manno-heptose is added to its derivative ADP, which is synthesised from sedoheptulose 7-phosphate in four steps. The genes *gmhA* and *gmhD* code for enzymes in the first and last steps. The G+C content of the *gmhA*, *gmhD* and *waaE* genes are 51%, 51% and 52.7%, respectively.

Kdo is transferred from CMP-Kdo and synthesised from arabinose-5P and PEP by a three-stage pathway[9]. Two of the genes in this process, namely, *kdsA* and *kdsB*, are well-characterised and have G+C contents of 51.6% and 52.7%, respectively.

The gene *waaA*, which is located in the *waa* cluster, codes for a bifunctional Kdo transferase that adds residues from 2 Kdo. These genes have a G+C content of between 51% and 54%. Several of the modifications of lipid A are regulated by the concentration of Mg^{2+} through the regulon *phoP-phoQ* [25].

PhoP-PhoQ is a two-component system that regulates virulence through adaptation to limited magnesium environments and regulates numerous cellular activities in gram-negative bacteria. This regulon consists of an external membrane sensor, PhoQ, and a cytoplasmic regulator, PhoP, and is activated by the acidic pH and by certain antimicrobial

peptides (APs). PhoP-PhoQ is repressed by millimolar concentrations of magnesium and calcium. PhoQ senses the concentration of magnesium and of APs throughout a periplasmic domain, which undergoes a conformational change when it is joined to these compounds and results in autophosphorylation. The activation of the PhoP-PhoQ system may allow for the activation or repression of 40 genes [26].

The regulon PrmAB of *Salmonella* is also a two-component system coded in the operon *pmrCAB*, which has protein products that include a phosphoethanolamine phosphotransferase (PmrC), a response regulator (PmrA) and a kinase sensor (PmrB). It has been confirmed that PmrAB regulates more than 20 genes in *Salmonella*. However, several studies suggest that more than 100 genes show activity[27]. One of the primary roles of the activation of PmrAB is the modification of LPS, such as the addition of Ara4N to lipid A, which, as explained below, impacts the susceptibility to some antimicrobial agents and the addition of phosphoethanolamine (pEtN) in the core of the LPS [26].

7.2. Regulation of the biosynthesis of the core

In *E. coli*, *Salmonella* and *K. pneumoniae*, the genes involved in the biosynthesis of the core are grouped on the chromosome. These loci code for the activities required in the assembly of the outer core and also code for the transferases necessary for the synthesis of the inner core. In *E. coli* and *Salmonella*, the *waa* locus (also called *rfa*) is formed by three operons and is located between the genes *cysE* and *pyrE*. The operons are defined by the name of the first gene in each transcriptional unit, such as *gmhD, waaQ* and *waaA* (**Figure 3**) [28].

Genes in the *waa* operon code for all of the transferases that assemble the core, including the gene that codes for the enzyme for the last step of the synthesis of ADP-L-Glycero-D-manno-heptose and the gene of the ligase of the O antigen, *waaL*. In addition, the *waa* operon contains the gene *waaA*, which codes for a bifunctional transferase Kdo.

The operon *gmhD*, which is located on the extreme 5′ end of the *waa* cluster, contains *gmhD, waaF, waaC* and *waaL*. The genes *gmhD-waaFC* are required for the biosynthesis and transfer of L, D-heptose. GmhD catalyses the last reaction of the synthesis of ADP-L-Glycero-D-manno-heptose. WaaC is the transferase for Hepl, and WaaF is a putative transferase of Hepll. WaaL is the ligase that bonds the O polysaccharide to the lipid A and core.

The transcription of the operon *gmhD* in *E. coli* K12 is regulated by heat shock promoters, indicating a requirement for the heptose domain of LPSs for growth at high temperatures [23,28,29].

The central operon *waaQ* contains 10 genes that are necessary for the biosynthesis of the outer core and for the modification of the core. WaaQ is the transferase for HepIII. The proteins WaaG, WaaO and WaaR are transferases for Glcl, Glcll and GlcIII, respectively, and WaaB is the transferase for the GaL residue. WaaP and WaaY are involved in the phosphorylation of the residue of heptose, whereas the functions of WaaU, WaaS and WaaZ are still not clear. In isolates of *E. coli* with the R1 and R4 cores, this operon contains the structural gene of ligase *waaL*, which must be produced for the union of the O polysaccharide with the complete core.

The *waaQ* operon is preceded by a JUMPStart sequence (Just Upstream of Many Polysaccharide-associated gene Starts), which includes a conserved region of 8 bp that is known as *ops* (operon polarity suppressor). The expression of the cluster for the biosynthesis of the core is regulated by the protein RfaH and also in response to thermal shock. RfaH is homologous to the factor NusG, which regulates the expression of the operon of hemolysin, genes of the polysaccharides of the capsule and genes for the transfer of the F plasmid. Regulation by RfaH occurs at the level of the polymerisation of the mRNA and depends on *ops* sequences that act in *cis*, as in the case of the operon *waaQGPSBIJYZK*, which includes 10 genes of the cluster of the core of LPS [22,30].

The transcript *waaA* contains the structural gene *waaA* (formally called *kdtA*), which codes for the transferase Kdo and a "non LPS" gene that codes for the adenylyltransferase pantetheine (*coaD*, formally *kdtB*) [31].

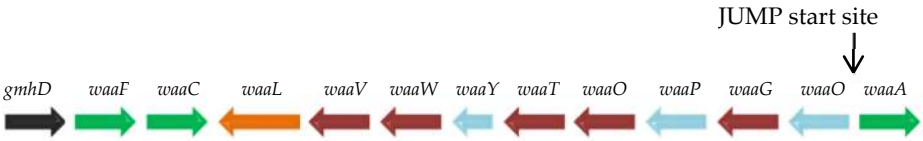


Figure 3. Structure of the genes involved in the synthesis of the core of *E. coli* R1. The genes of the glycosyltransferases that form the bonds of the inner core are shown in green. Those genes of the enzymes that modify the structure are in red, and the glycosyltransferases of the outer core are in blue. In orange are genes that modify the ligase enzyme.

7.3. Regulation of the biosynthesis of the O polysaccharide

The genes involved in the biosynthesis of the O antigen are generally found in the chromosome in the cluster of the O antigen or *rfb*. These genes have a lower GC content than the average for genomes, between 30% and 40%, which provides evidence that these genes have been acquired through lateral inter-species transference [24].

The genes that code for the proteins that participate in the synthesis of the O antigen form three main groups: a) proteins involved in the biosynthesis of the precursors of nucleotide sugars of the O antigen; b) protein glycosyltransferases, which sequentially transfer various precursor sugars to form an oligosaccharide of a lipid carrier, undecaprenyl phosphate (UndP), which is located in the cytoplasmic face of the internal membrane and c) genes for the processing of the O antigen, which are involved in the translocation through the membrane and polymerisation (**Figure 4**) [32,33]. A fraction of O antigens includes acetyl-O groups, and others include residues; therefore, in the corresponding clusters, the transferases for them are coded. The differences among the many forms of the O antigen are due to the genetic variation in the cluster of the O antigen. The genes for the initial steps, which are also involved in conserved functions, do not duplicate in the cluster of the O antigen [24].

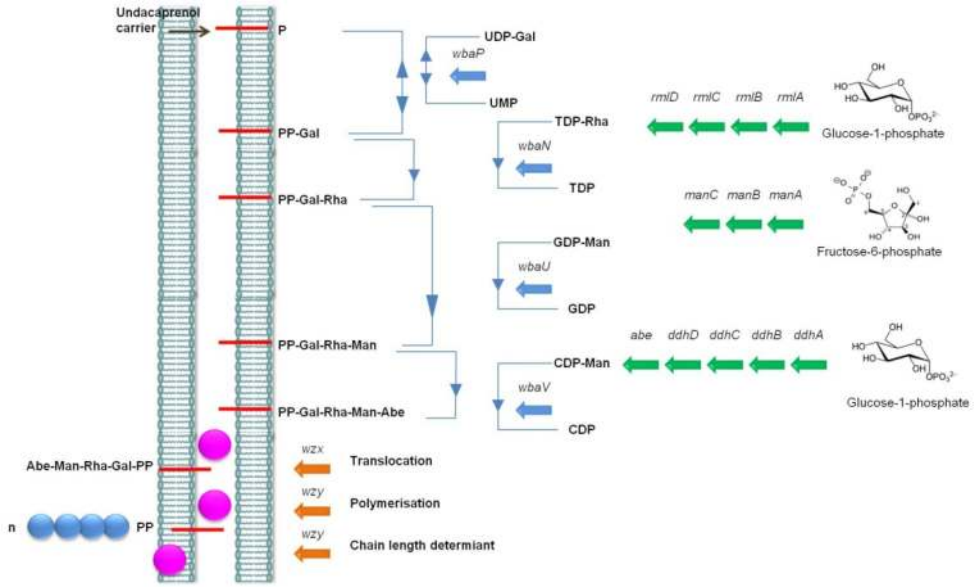


Figure 4. The synthesis of the O antigen involves three types of genes: a) genes related to the biosynthesis of precursors of sugar (green boxes), whose protein products perform their function in the cytoplasm; b) genes for glycosyltransferases (blue boxes), whose corresponding proteins transfer nucleotide sugars in the UndP lipid to form the O unit. This process occurs on the cytoplasmic side of the internal membrane and c) genes for the assembly of the O antigen and its exportation (orange box). Modified from [38].

In *E. coli* and *S. enterica*, the genes involved in the synthesis of the O antigen are typically found among the constituent genes *galF* and *gnd*. In *P. aeruginosa,* these genes are found among *himD* and *tyrB* [15]). In *V. cholerae*, O antigen synthesis genes are found among *gmhD* and *rjg* [34], and in *Yersinia* spp., these genes are found among *hemH* and *gsk.* Some exceptions, such as the polysaccharide O54 of *S. enterica*, which is a cluster that is coded in a plasmid [35] (**Figure 5A**).

The genes of the biosynthetic pathway of the three precursors of the nucleotides of sugar are grouped within the genetic cluster of the O antigen. *manB and manC* code for enzymes that convert mannose-6-P into GDP-mannose. The operon *rmlABCD* codes for enzymes that form TDP-rhamnose from glucose-1-P. *ddhABCD* and *abe* code for enzymes to make CDP-abequose from glucose 1-P. UDP-Gal is used in other pathways and is synthesised by conserved enzymes. The transferase galactose, which is coded by *wbaP*, initiates the synthesis of the O units by transferring galactose phosphate from UDP-Gal to UndP.

The transferases coded by *wbaZ*, *wbaW* and *wbaQ* are positioned over two residues of mannose and one of rhamnose before the residues of mannose, which is acetylated by WbaL. The residue of abequose is bonded to acetyl rhamnose by WbaR to form a complete O unit. The flippase of the bond of the O antigen is coded by *wzx* and is responsible for

moving the O unit bonded to UndP through the internal membrane before the polymerase Wzy exerts its action over the long-chain O antigen.

The majority of the O antigen operons are constitutively expressed and are preceded by a sequence of 39 bp, known as JUMPStart **(Figure 5B)**.This sequence includes two elements known as *ops*, which are involved in the recruiting of elongation factors during the transcription. The JUMPStart region is controlled by RfaH, which acts as a positive regulator for the genes involved in the outer core of LPS and also increases the expression of the locus *rfb* [1,18,19.]

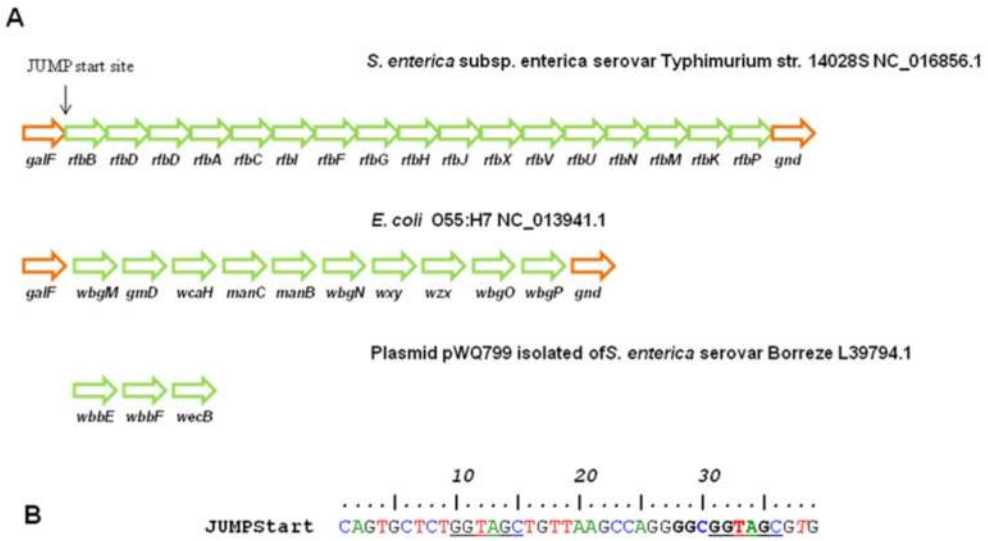


Figure 5. A. The location of the genes involved in the synthesis of the O antigen in several species of *Enterobacteriaceae*. **B.** The JUMPStart sequence of *S. flexneri* strain IB1833**.** The *ops* sequence is indicated in bold for eight base pairs. A residue of thymine that is highly conserved in the far 3′ is indicated by cursive text, and repeated sequences are underlined. Accession number: U118168.1.

8. Variability of the LPS

In bacterial pathogens, the most variable structures are those that are expressed on the cell surface. Intra-species genetic individuality has inheritable traits that may be observed in a sample of individuals. The genetic variability is derived from the combination of a certain number of genes, which each exists as a family of alleles that differ in structure and function. An example is the family located in the biosynthetic locus of the O antigen, and an example of this phenomenon at the population level is the hypervariability in the structure of this biomolecule [39]. Among the components of the surface of the gram-negative bacteria, LPS is the primary constituents of the external membrane, which is a heterogeneous surface that is significantly involved in the process of the microorganism adapting to its environment.

LPS primarily consists of conserved segments, such as lipid A and the core, and secondarily consists of a hypervariable segment, which is the O antigen. The conserved domains of the LPS are shared regions among bacterial species, which intervene in the development and in the survival of the bacteria. The O antigen may display modifications such alterations in the length of the oligosaccharide chain and changes in the surface composition and in the chemical configuration, due to the addition of glycosyl or fucosyl groups or even non-hydrocarbonated substitutes, such as acetyl or methyl groups, which could affect the cellular structure [40].

8.1. Intra-species variability in the composition of the O antigen

The heterogeneity in the expression of LPS may provide a medium to discriminate among the bacterial species. This heterogeneity is responsible for the well-known ladder profile that can be detected in silver-stained SDS-PAGE gels (**Figure 6).** This method is used to determine the number and repeated units of oligosaccharides that constitute the O antigen, which has been useful in epidemiological studies [41]. The smooth strains contain the entire LPS, whereas the semi-rough strains have a subunit of the O antigen, and the rough strains lose the subunits of the O antigen.

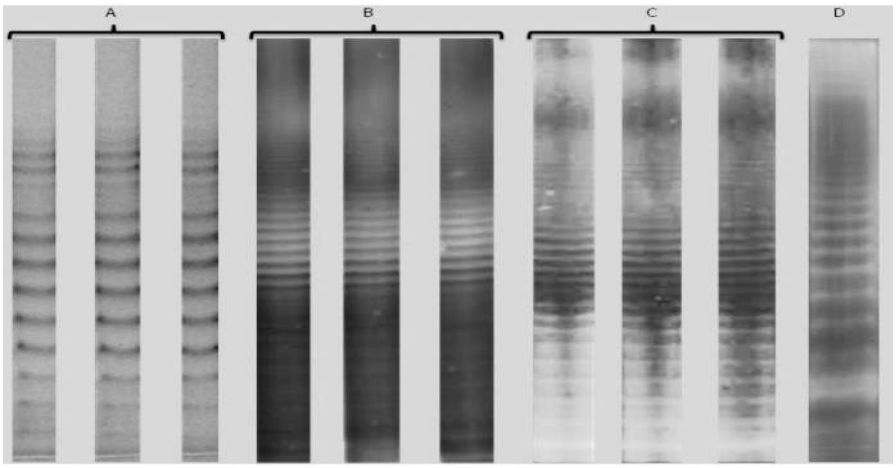
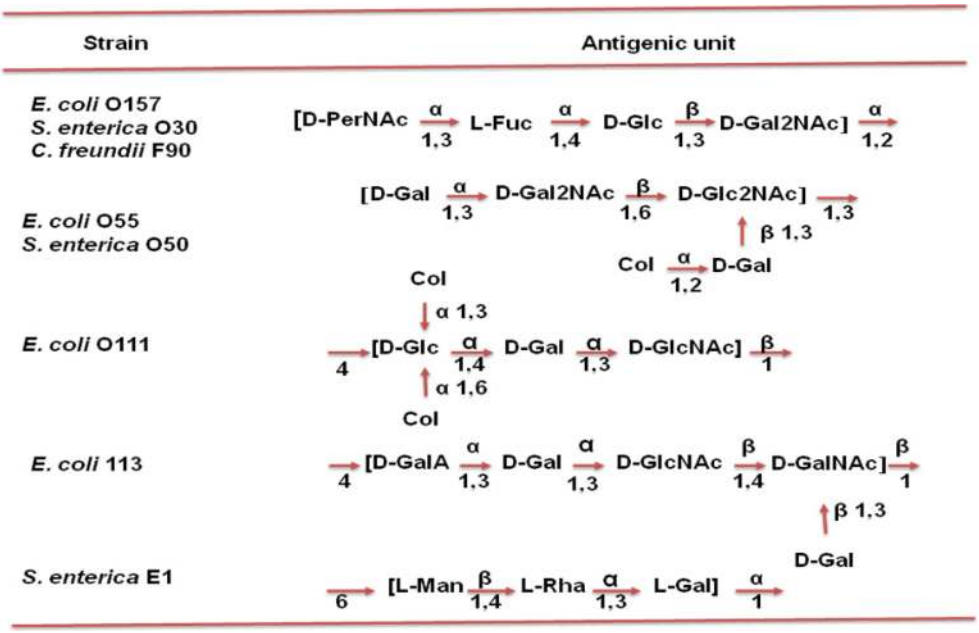


Figure 6. LPS profiles in SDS-PAGE. The smooth forms of LPS display a ladder profile that is strain-specific. **A)** *C. freundii* E9750; **B)** *S. senftenberg* 74210; **C)** *E. coli* O157:H7; **D)** *S. marcescens* biotype TC.

In **Table 1**, examples are presented of various sequences from different genera and species, which mark the variability of intra-species and inter-species LPS that impedes the immune response control of bacterial infections, among other issues.



D-Col, D-colitose; L-Fuc, L-fucose; D-Gal, D-galactose; D-Gal2NAc, 2-*N*-acetylgalactosamine; D-Glc, D-glucose; D-Glc2NAc, D-2-*N*-acetylglucosamine; D-Per, D-perosamine; D-Per4NAc, D-4-*N*-acetylperosamine.

Table 1. Examples of different structures of the bacterial O antigen.

An increase in temperature during microbial growth causes changes in the concentration of carbohydrates in LPS, which modifies their composition [42]. In a study on *in vitro* passages of the *C. freundii* E9750 strain cultivated under different temperatures, variability was observed in the ladder profiles of the O antigen, in the concentration of carbohydrates and in the agglutination reactions with the anti-O serum of *C. freundii* E9750 and *Salmonella senftenberg* 74210 [43]. According to the ladder profiles of the isolates of *C. freundii* E9750, six profiles were distinguished: A (control strain), B, C, D, E (immunoreactive isolates) and F (isolates that had lost the immunoreactive chain). These profiles were generated according to their similarity. Each LPS profile displayed a typical ladder profiles (**Figure 7).**

The different profiles from the isolates obtained from the sub-cultures of *C. freundii* E9750 were out of phase with respect to the control. This result may be explained by variation in the number of oligosaccharide units present in the O antigen. However, when the length of the chain is increased, the differences are more marked, and the bands of the ladder profiles are out of phase, as Lawson et al., report with strains of *S. enterica* serovar Typhimurium [41]. There are other reports in the literature on the variability of the length of the O antigen chain as a response to change in temperature, as in strains of *P. aeruginosa* and enteric bacteria [44,45].

Several isolates of *C. freundii* E9750 displayed cross-reactivity in agglutination tests with the anti-O serum of *S. senftenberg* 74210, which suggests changes in the conformation of the

epitopes that may be associated with the addition of glucosyl groups or residues derived from N-acetyl [46].

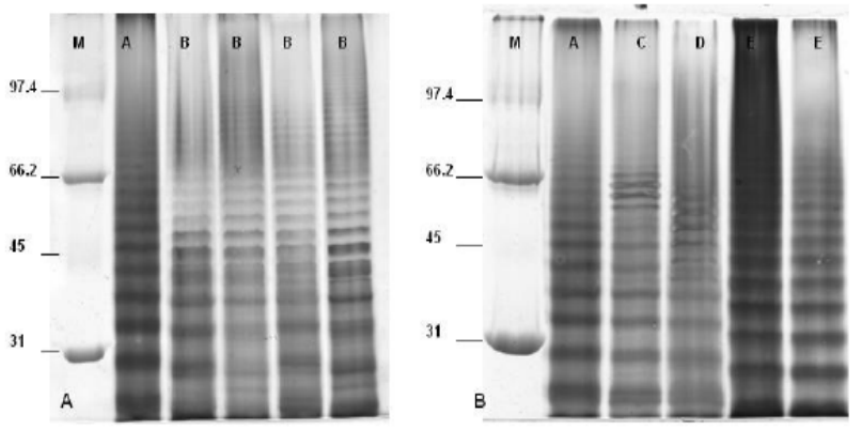


Figure 7. LPS profiles in SDS-PAGE stained with silver. Profiles of colonies of *C. freundii* E9750. A) isolates at a temperature of 42°C; B) isolates at a temperature of 37°C. M= weight markers.

The isolates of *C. freundii* E9750 that display variability suggest heterogeneity in the composition or conformation of the epitopes of the O antigen. The cross-reactivity of the isolates of *C. freundii* and *S. senftenberg* 74210 is associated with the specificity of the epitopes of the O polysaccharide that these species share. The addition at O antigen of glucosyl groups or derived N-acetyl residue could be involved in these cross-reactions.

8.2. Heterogeneity of populations and phase variation

Phase variation is used by various bacterial species to generate diversity within a population. Phase variation is a process of change in the expression of the epitopes of the cellular surface of the bacteria [19]. Bacterial cells may phenotypically vary even within a clone population, which allows them to adapt to their environment or even to evade the immune response of the host. Phase variation is a phenomenon that generates phenotypic heterogeneity within a population by means of gene regulation, which changes genes from a state of expression in which they are "turned on" to a state of non-expression in which they are "turned off". The state of expression is inheritable, reversible and affects the same phenotype.

Antigenic variation is referred to as the expression of an alternative form of an antigen of the cellular surface, such as polysaccharides, lipoproteins and type IV pili, which at the molecular level, share characteristics with the phase variation mechanisms. During this adaptation process, the bacteria display reversible phenotypic changes as a result of genetic changes or epigenetic alterations in a specific locus. The mechanisms which allow for phase variation are: genetics (Slipped-strand mispairing, recombination) and epigenetic (DNA methylation) [47,48].

a. Genetic mechanisms

Numerous studies have been performed to reveal the genetic basis of the variation of the O antigen. In certain cases, the variability in the expression of the genes is regulated by elements in *cis*, which cause changes in the composition of the structure of the antigens of the bacterial surface. Certain pathogens change the structure of the O antigen through the acquisition of phage genetic material followed by recombination processes [7].

i. Slipped-strand mispairing

One of the mechanisms that regulate phase variation at the molecular level is the slipping of one of the DNA strands, which causes a mispairing between the daughter strand and the parent strand during the replication of the DNA. This process is known as slipped-strand mispairing (SSM). The genomic sequences susceptible to SSM are those which contain short repetitions, microsatellites or a variable number of in tandem repetitions, which may cause a change in the expression of genes at the level of the transcription processes or translation according to the location of the repeated sequence in relation to the promoter and the codifying sequence [49]. At a transcriptional SSM, this may lead to the activation or deactivation of the promoter region of the target gene, as occurs in *H. influenzae (hif*A/B). At a translational level, SSM may affect the codifying region, as for example, with the genes involved in the biosynthesis of the LPS of *H. influenzae* and *Neisseria* spp. [50].

Within the genome of *H. pylori*, certain loci have been identified with repeated sequences of a single nucleotide or a pair of nucleotides. Several of these repetitions are within the open reading frames (ORFs) (**Figure 8)**. In the transcription process, the mispairing between nucleotides, when one of the strands of DNA slips over another chain, causes the "gain" or "loss" of a unit in the reading frame, which leads to the loss of the start codon or mutations in the proteins. Therefore, SSM increases the genetic variability of *H. pylori.* Similar repetitive sequences have been found in other microorganisms, such as *H. influenzae* [51].

One group of genes that generate phase variation are those that code for enzymes that intervene in the biosynthesis of LPS, which may cause variants of the gene product in the same bacterial population. The LPS of the majority of *H. pylori* strains contains complex carbohydrates known as the Lewis antigen. Type 1 (Le^a,Le^b) and type 2 (Le^x,Le^y) Lewis antigens are epitopes of fucosylated oligosaccharides. At least 80% of the strains of *H. pylori* express type 2. Some research on the antigenic determinants involved in the biosynthesis of the Lewis antigen have allowed for the identification of the fucosyltransferases (FucTs) that are involved in the formation of these antigens [19].

The genes that code for the FucTs have elements in *cis* that are differentiated by containing poliA and poliC sequences of different lengths that mediate SSM. The size of these sequences regulates the activation and deactivation of the genes of the FucTs. However, in some cases, such as in *H. pylori* UA948, the inhibition of the expression of *futB* is due to mutations outside of the hypervariable region (the elimination of 80 nucleotides in the promoter region) [52].

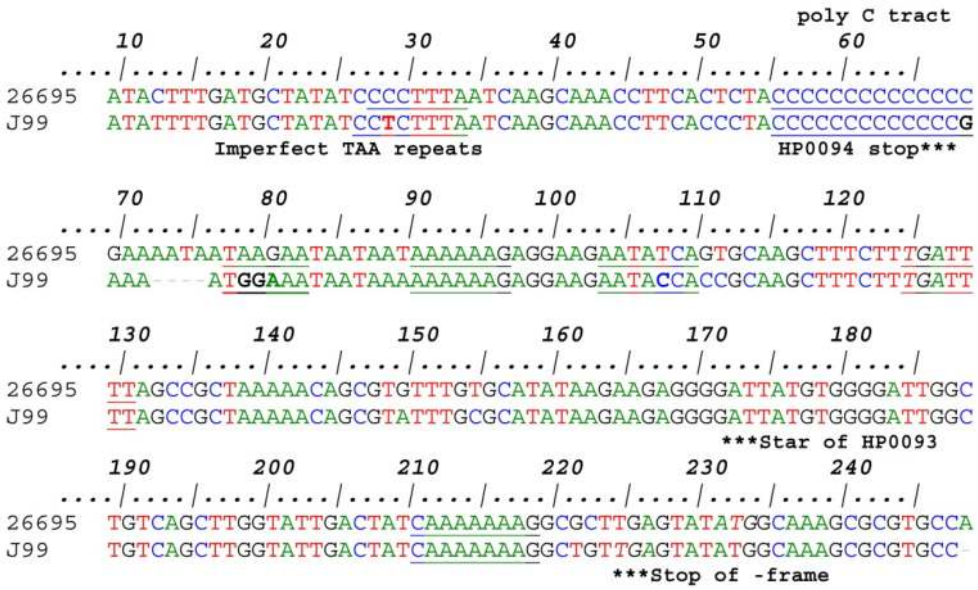


Figure 8. The nucleotide sequence of the central region of the Hp *fuc*T2 gene. The sequences show the characteristics (simple repetitions) responsible for the ORF in the *H. pylori* J99 strain and the 26695 type variant. Due to the number of different repetitions of the residues of poli C, start sequences of an ORF of *fucT2* of the 26695 strain are found in the TGA stop codon (marked with asterisks) shortly after reading 1 (HP0093), which is the same as the marker of the *fuc*T J99 reading. The three supposed motifs X XXY YYZ are highlighted in bold and are underlined.

The α 1,2-fucosyltransferase (FutC) catalyses the addition of fucose in the conversion process of Le^X to Le^Y. Sanabria-Valentín et al., through *in vitro* and *in vivo* studies, confirmed the main function of *futC* slipped-strand mispairing in the variation of the Le antigen [53].

The *futC* gene includes an internal Shine-Dalgarno type sequence and a heptamer (AAAAAAG) followed by a loop structure. During translation, when the ribosomes are in the heptameric sequence of the mRNA, a phase shift occurs in the reading frame. The presence of Shine-Dalgarno type sequences and the loop structure accelerate the translation process by an interaction with the ribosome components [19].

ii. Phage recombination: seroconversion

The O antigen is a determinant of the virulence necessary for the pathogenicity of *S. flexneri*. The O antigen of *S. flexneri* is called the Y serotype and consists of repeated units of a tetrasaccharide of N-acetylglucosamine-rhamnose I-rhamnose II, rhamnose III, which forms the structure of the vertebral column of the polysaccharide unit of all the serotypes of *S. flexneri*, except the 6 and 6a serotypes. There are 13 serotypes, which are differentiated by the addition of glucosyl groups or acetyl residue to the different sugar molecules in the tetrasaccharide unit [54,55].

The temperate phages of *S. flexneri* play an important role in the processes of seroconversion (antigenic variation). The bacteriophages SfV, SfII, SfX, the cryptic prophages SfI and SfIV code for the *gtr* genes, which are the proteins involved in the glycosylation of the O antigen. When these phagic elements lysogenise, a conversion of Y serotype strains into 5a, 2a, X, 1a and 4a serotypes, respectively, occurs (**Figure 9**).

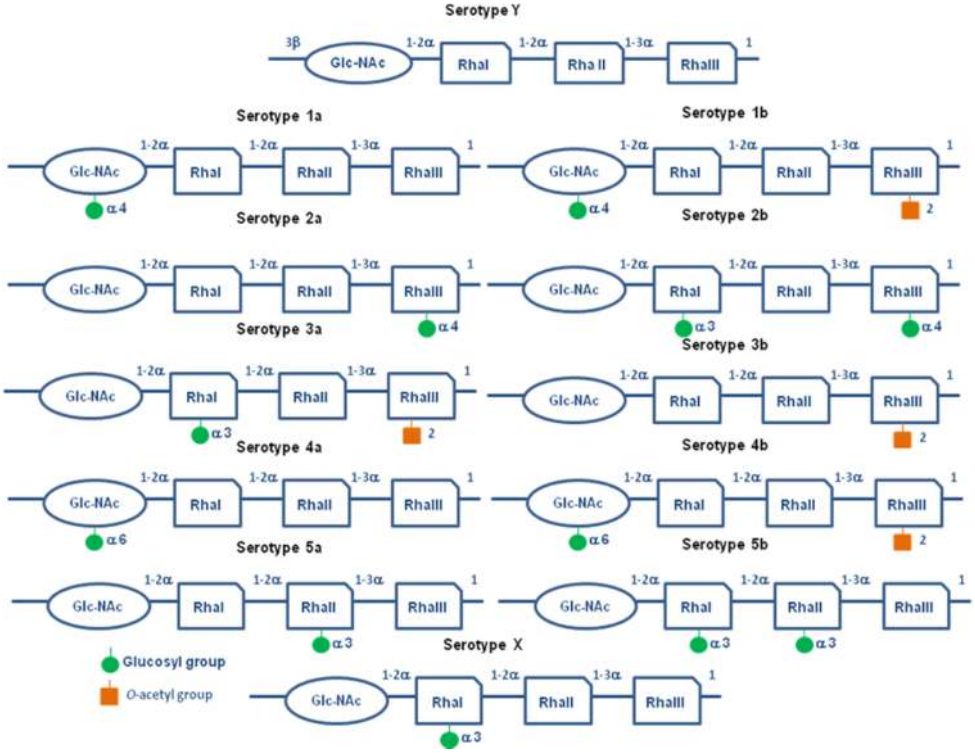


Figure 9. The chemical composition of the different serotypes of *S. flexneri*. The serotype Y formed by repeated units of tetrasaccharide N-acetylglucosamine-rhamnose I–rhamnose, II–rhamnose III. The serotypes are differentiated by the bonding of the glucosyl or the acetyl group. Adapted from [54].

The bacteriophage codes for an acetyltransferase and produces a conversion to the 3b serotype. The lysogenisation of the SfV bacteriophage produces modifications of the type V O antigen, which involves the addition of a glycosyl group through a bond of α 1,3 to the rhamnose II of the repeated tetrasaccharide unit. Similar to other phages that intervene in the glycosylation process, the genes involved in the conversion of serotypes are located immediate downstream from the *attP* locus, which is preceded by the genes *int* and *xis*. These phages are inserted into the *thrW* locus of the host [56].

The *gtr* genes of the temperate phages that code the glycosyltransferases are located in the genome of the phage, downstream from the *attP* locus. These genes are found in a cluster of three genes: *gtr*A, *gtr*B and *gtr* (type), which are cotranscribed. *grt*A and *grt*B are

homologous and are interchangeable among the serotypes of *S. flexneri*. The Gtr (type) protein is specific for the formation of the glucosyl bond in a particular sugar molecule of the O antigen [54].

It has been suggested that GtrB catalyses the transfer of glucose from UDP-glucose to bactoprenol phosphate to form UndP-β-glucose in the cytoplasm. This molecule is subsequently translocated by GtrA in the periplasm before the glucosyl residue is joined by Gtr(type) for the growth of the O antigen unit [57].

The genes *gtr*V and *grtX* code for the glycosyltransferases GtrV and GtrX, respectively, which are membrane proteins that catalyse the transference of glucosyl residues through the bonding of the 1,3 rhamnose II and rhamnose I of the O antigen unit. This intervenes in the conversion of the serotype of *S. flexneri* from Y to the 5a serotype and the X serotype, respectively. GtrlV adds glucosyl residues to N-acetylglucosamine of the repeated unit of the O antigen through an α 1,6 bond, converting the Y serotype into the 4a serotype [57,58].

b. Epigenetic mechanisms: DNA methylation

The term epigenetic is defined as "inheritable changes in genetic expression that occur without alterations in the DNA nucleotide sequence". Thus, an epigenetic mechanism may be understood as a complex system to use the genetic information selectively by activating and deactivating various functional genes. Epigenetic modifications may imply methylation of cytosine residue in the DNA. DNA methylation has been observed in various bacterial species. In bacteria, methylation is part of a defence mechanism to reduce the amount of horizontal genetic transference among species. DNA methylation constitutes an epigenetic marker that identifies the template strand during the replication of the DNA. Generally, the methylation of the regulatory elements of genes, such as promoters, enhancers, insulators and repressors, suppresses this function [59].

The modifications of the O antigen that may affect the serotype are related to those that contain the operon that code for the glycosyltransferases (*gtr*).

Within a clone population of *S. enterica* serovar Typhimurium, the lysogenic phage P^{22} may lead to variability of the O antigen. The phase variation of the gtr (glycosylation of the O antigen) indirectly contributes to the diversity of the serotypes of *Salmonella*. The cluster that codes for the glycosyltransferases consists of three genes: *gtrA* codes for a membrane protein, *gtrB* codes for a glycosyl translocase and *gtrC* codes for the glycosyltransferase, which mediates the bonding of glucose to the O antigen [58].

Through studies based on the analysis of gene expression, the presence of mutations, the level of DNA methylation and the *in vitro* interaction of DNA-proteins, Broadbent et al., demonstrated that the Dam methyltransferase proteins together with OxyR regulate phase variation at the level of the gtr_{P22} promoter in *S.enterica* serovar Typhimurium. OxyR is an activator or a repressor of the *gtr* system, which depends on the alternative side (GATC sequences) to which OxyR bonds in the gtr_{P22} regulatory region (**Figure 10)**. The bonding of OxyR is inhibited by the methylation of the Dam target sequence, and the state of expression of the system is inheritable [60].

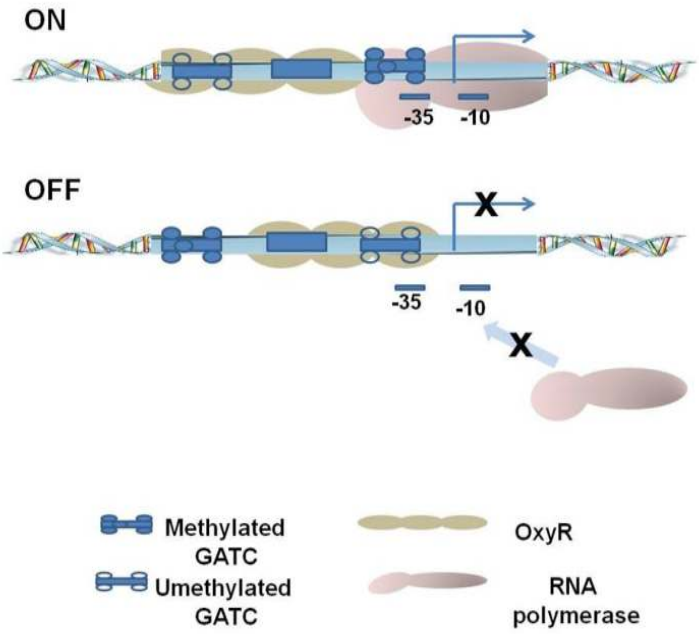


Figure 10. Model of the phase variation of the regulatory region of the *gtr* operon. Illustration of the interaction of DNA proteins in the regulatory region of the *grt* P22 operon, which consists of methylation and demethylation in the GATC sequence in the activated and deactivated phases. Adapted from [60].

Understanding the variation of the LPS structure is important because the composition and the length of the O antigen chain may be an indicator of the virulence, and this characteristic often differs within a single bacterial strain [7].

9. The importance of the variability of the O antigen of LPS

The modifications that are present in the O antigen and that cause its variability play an important role in infections by gram-negative bacteria, given that the modifications may influence adherence, colonisation and the ability to evade the host's defence mechanisms.

9.1. The role of the variation of LPS in the immune response.

LPS activates not only the innate immune response but also the adaptive response. The first contact that LPS has with the immune system is with lipid A, which is recognised by the receptors involved in the innate immune response, while the structure of the O antigen participates in the adaptive response (synthesis of antibodies). LPS is a potent stimulator of the cells of the immune system, given that it induces the production of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 1β (IL-1β) and acute phase proteins.

Although the variability is greater in the O antigen within LPS, there is also variability within lipid A. This variability is due to the length of the chains and the saturation of its acyl groups. Because these groups are strong immunostimulants, both the changes in the number of their chains and the presence of phosphorylations within the structure of the lipid A may influence its biological activity [61].

Lipid A is the structure of LPS that is recognised by the TLR4 receptors, which are part of the so-called Toll Like Receptors (TLRs), which are expressed by cells of the innate immune system and are stimulated by pathogen-associated molecular patterns (PAMPs). The stimulation of the LPS in certain cells of the monocyte-macrophage lineage, lymphoid cells and even cells that are not part of the immune system, such as epithelial cells, endothelial cells and vascular cells, occurs with the participation of other molecules, such as LPS binding protein (LBP), CD14 and MD-2. The transduction signals of TLR4 are divided into MyD88-dependent and MyD88-independent (also called TRI-dependent) groups. These signals may be regulated at various levels. For example, the RP105 and SIGIRR (Single immunoglobulin IL-IR related molecule) molecules inhibit the start of the signalling cascade [62].

Recognition through TLR4 is crucial for the control of infection, but changes in the signalling pathways may cause sepsis or evasion of the pathogen. The importance of signalling via TLR4-MD2 in response to gram-negative pathogens make this pathway an alternative to search for therapeutic targets not only for infectious diseases but also for other diseases with inflammatory aetiology, such as cancer, atherosclerosis, asthma and autoimmune conditions.

Antagonists of TLR4-MD2 have been identified, and several of these are based on the lipid A structures and other inhibitor molecules [63–65]. The intention is to use this type of antagonist therapy to treat septic shock. Additionally, several TLR4 antagonists primarily those that activate the TRAF (TNF receptor- associated factor) or TRIM (Tripartite motif) pathways have been proposed as adjuvants.

However, certain pathogens have the ability to modify the structure of lipid A and its detection by the host. For example, some isolates of *P. aeruginosa* are capable of modifying the structure of lipid A into a penta-acylated moiety, which does not activate the TLR4 and allows the immune response to be evaded. Other isolates of *P. aeruginosa* colonise respiratory pathways of patients with CF during its adaptation, producing hexa-acylated structures that are highly pro-inflammatory [61].

The large variability that the O antigen displays allows for the existence of various clones within a single species, which offers a selective advantage in the niche occupied by this clone and is precisely the interaction between the O antigen and the immune system that permits this advantage.

Many pathogens have the capability of varying the antigens that are attached to their surface and therefore can vary their antigenic composition. This variation is typically

mediated by the regulation of the expression of genes. By varying their antigenicity, the pathogens have a greater ability to evade the immune response of the host, and this variability makes it more difficult to design vaccines for these pathogens [66].

The O antigen is considered to be highly immunogenic and induces the production of antibodies that may activate the complement pathway, either through the classic pathway or an alternate pathway, which leads to cellular death or phagocytosis. Certain modifications in the oligosaccharide chain of the O antigen may alter the interaction of the complement pathway. Several O antigens of pathogens are similar to host molecules and facilitate invasion through mimicking in the host; for example, O antigens of the LPS of *H. influenzae* and of *N. gonorrhoeae* mimic epitopes of glycosphingolipids [67].

The mimicking property may also serve to evade the immune system, as is the case of *H. pylori.* The chains of the O antigen that contain the surface of the LPS of *H. pylori* express Lewis antigens, mainly Le^x and Le^y, although some isolates may contain other antigens, such as Le^a, Le^b, Le^c, Sialyl-Le^x and H-1, in addition to type A and B blood groups [68].

The expression of Lewis antigens and their fucosylation have biological effects in the pathogenesis of this bacterium. The O antigen of *H. pylori* exhibits molecular mimicking with the Lewis antigens of the host within the gastric epithelium. The expression of the Lewis antigens is subject to phase variation, given that the regulation of the glycosyltransferase genes is regulated by SSM, which in the O antigen structure promotes variations among the strains. The antigenic mimicking is essentially involved in the evasion of the immune system and gastric adaptation. Several studies show that mimicking also plays a role in the colonisation and adhesion of Le^x of the bacteria with the galectin-3 of a gastric receptor.

Moreover, *H. pylori* is capable of evading the binding effect of surfactant protein D, which is expressed in the gastric mucosa and is a component of the innate immune response [69]. This microorganism impedes the bonding of the surfactant protein through the variation of its LPS. This phenomenon is associated with changes in the fucosylation of the O antigen chain. In addition, the expression of Lewis antigens affects both the inflammatory response and the polarisation of the T cells that are triggered after an infection. Because it is a chronic pathogen, several studies have shown that *H. pylori* may induce anti-Lewis auto-reactive antibodies, which enable the gastric mucosa to be recognised and contribute to the development of gastric atrophy [70].

9.2. Changes in the LPS related to resistance

The hydrophobic antibiotics that reach the interior of the cells due to the permeability of the external membrane are aminoglycosides, macrolides, rifamycins, novobiocin, fusidic acid and cationic peptides. The tetracycline and the quinolones use pathways that are mediated by lipids and porins. The central region of the LPS is important because it provides a barrier against hydrophobic antibiotics and other components; isolates that express a long LPS have intrinsic resistance to these factors [71].

The polymyxins, which include polymyxin B and colistin (polymyxin E), belong to a group of natural antimicrobials that are found in eukaryotic cells; this group is known as the cationic antimicrobial peptides. The polymyxins are active against gram-negative pathogens, such as *P. aeruginosa, Acinetobacter baumanii, Klebsiella* spp., *E. coli* and other *Enterobacteriaceae* [71–73].

The LPS has a negative charge and provides integrity and stability to the external membrane of the bacteria. Polymyxin has a positive charge, displacing the Mg^{+2} or Ca^{+2} and bonding to LPS, which as a consequence, destabilises and destroys the internal and external membrane [74].

Gram-negative bacteria may develop resistance to colistin and polymyxin B. The most important mechanisms involve modifications in the external membrane through changes of the LPS. The modification of the LPS occurs with the addition of 4-amino-4-deoxy-L-arabinose (Lara4N) to a phosphate group in lipid A. This addition causes an decreases in the negative charge of the lipid A, which decreases the affinity of the positively charged polymyxins [68–70].

The biosynthesis of LAra4N is mediated by the regulatory systems PmrA/PmrB and PhoP/PhoQ [26]. One of the primary roles of the activation of PmrAB is the modification of the LPS. These modifications include additions of Ara4N and pEtN to the lipid A and of pEtN to the core of LPS. The modifications mask phosphate groups with positive charges, thereby affecting the electrostatic interaction with certain cationic compounds. The biosynthesis of LAra4N depends on the genes of the operon of resistance to polymyxin, which is known as *arn*. This operon includes the genes *pmrHFIJKLM* [71–73].

10. Conclusion

In the bacterial pathogens, the most variable structures are those expressed in the cell surface. LPS is one of the principal antigenic structures of cell surface of gram-negative bacteria. A great variability in LPS has been demonstrated and principally in O antigen of gram-negative bacteria. This variability is present not only in the longitude of the oligosaccharide chains but also in the composition and structure of LPS.

Many of the functions of O antigen are associated to the longitude of the chain and to the variability of its structural features. This variability could affect the function, physical and chemical properties as well as the target site of LPS and determines the changes in the virulence of the microorganism that favor its adaptation to fluctuating enviroment which in many occasions are hostile to the microorganism and permit its evasion of the immune response of the host. The variation in O antigen structure has demonstrated that its composition and the longitude of its chain could be biological markers of virulence and this characteristic could differ within the same bacterial strain. The variability of LPS could derive from adaptations that involve associated changes to the synthesis of this molecule. The antigenic variability could occur by means of genetic and epigenetic mechanisms. The lost or gain of genes associated to variability of LPS is due to the events of genetic material

interchange produced by lateral transference of genes which leads to strain selection with new characteristics and the evolution of the bacteria by modification of this structure.

One of the most important aspects of LPS function is its participation as immunogenic molecule and its role in bacterial classification based on O antigen and its variability. In general, it is seen that the modifications of O antigen play an important role in the process of infection including the adherence, the colonization, and the ability to evade defensive mechanisms of the host especially the innate resistance.

The study of the events of variation of LPS and its effects on pathogenecity and virulence represents a field of study of great interest to understand bacterial physiology and its mechanisms of adaptation and evolution.

The immunogenicity and variability of O antigen confer to gram-negative bacteria an important characteristic for its serological typification. The 0-antigen is subject to an intense selection on the part of immune system, which could be the principal factor for the different forms in which it is presented. For this reason, the variability of O antigen has been an area of intense research.

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