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REVIEW

Bacterial Cell Surface Structures in *Yersinia enterocolitica*

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Abstract *Yersinia enterocolitica* is a widespread member of the family of *Enterobacteriaceae* that contains both non-virulent and virulent isolates. Pathogenic *Y. enterocolitica* strains, especially belonging to serotypes O:3, O:5,27, O:8 and O:9 are etiologic agents of yersiniosis in animals and humans. *Y. enterocolitica* cell surface structures that play a significant role in virulence have been subject to many investigations. These include outer membrane (OM) glycolipids such as lipopolysaccharide (LPS) and enterobacterial common antigen (ECA) and several cell surface adhesion proteins present only in virulent *Y. enterocolitica*, i.e., Inv, YadA and Ail. While the *yadA* gene is located on the *Yersinia* virulence plasmid the Ail, Inv, LPS and ECA are chromosomally encoded. These structures ensure the correct architecture of the OM, provide adhesive properties as well as resistance to antimicrobial peptides and to host innate immune response mechanisms.

Keywords *Yersinia enterocolitica* · Lipopolysaccharide · Enterobacterial common antigen · *Yersinia* virulence plasmid

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Historical Notes on the Discovery of *Yersinia enterocolitica*

Only two *Yersinia* species (at that time *Pasteurella*) *P. pseudotuberculosis* and *P. pestis* were known in 1934 when McIver and Pike isolated an unknown rod-shaped coccobacillus from facial abscesses of a 53-year-old farmer (McIver and Pike 1934). The patient, beside the facial ulcerations, suffered from bacterial infection of the lymphatic system manifested by engorgement of cervical lymph nodes. Originally, the isolate was identified as *Flavobacterium pseudomallei*, an etiologic agent of endemic melioidosis, commonly occurring in countries with hot and humid climate (Bottone 1997; Adler et al. 2009).

In 1939, Schleifstein and Coleman reinvestigated the coccobacillus of McIver and Pike and named it *Bacterium enterocoliticum* (Schleifstein and Coleman 1939, 1943). Moreover, they found out that *B. enterocoliticum* could be easily isolated from stool specimens of patients with acute enterocolitis. A year later, Van Loghem established a new bacterial genus: *Yersinia* Van Loghem (1944–1945). In 1964, Frederiksen merged the words "*Yersinia*" and "*enterocoliticum*" creating thereby the contemporary scientific name *Yersinia enterocolitica* (Frederiksen 1964).

Currently, the genus *Yersinia* consists of 17 bacterial species, among which only 3 are pathogenic in animals and humans, i.e., *Y. pseudotuberculosis*, *Y. pestis* and *Y. enterocolitica*. The remaining 14 species: *Y. aldovae*, *Y. aleksiciae*, *Y. bercovieri*, *Y. entomophaga*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii*, *Y. nurmii*, *Y. pekkanenii*, *Y. rohdei*, *Y. ruckeri*, *Y. massiliensis* and *Y. similis* are either regarded as avirulent (alternatively called "environmental-type" bacteria) or their presumptive pathogenicity has not been so far studied or confirmed (Hurst et al. 2011; Merhej et al. 2008; Murros-Kontiainen et al.

2011a, b; Sprague and Neubauer 2005; Sprague et al. 2008; Sulakvelidze 2000).

Characteristics of *Y. enterocolitica*

Yersinia enterocolitica are Gram-negative, rod-shaped, non-sporulating, facultatively anaerobic γ-proteobacteria that belong to family *Enterobacteriaceae*. An intriguing feature of *Y. enterocolitica* is that both motility and expression of specific virulence factors are temperature-regulated. *Y. enterocolitica* is a heterogenic species that includes both pathogenic and non-pathogenic strains that are distributed into different bio- and serotypes. Certain bio-/serotype combinations predominantly contain virulent strains that cause yersiniosis both in animals and humans. Representatives of *Y. enterocolitica* are widespread in nature, where they can be found in aquatic environments, soil and gastrointestinal tracts of many higher vertebrates, especially pigs (Bottone 1997, 1999).

Based on metabolic differences between *Y. enterocolitica* isolates, six biotypes have been established: 1A, 1B, 2, 3, 4 and 5. While the biotype 1A strains are considered apathogenic, strains of the remaining five biotypes are significant animal and human pathogens. Furthermore, biotype 1B strains are considered highly and biotype 2–5 strains less pathogenic for mice (Table 1) (Bhagat and Virdi 2007; Hudson et al. 2008).

Y. enterocolitica isolates are also distinguished serologically based on antigenic variation in lipopolysaccharide (LPS) O-polysaccharides (O-PS; O-antigen), capsules (K-antigens) and flagellae (H-antigens) (Table 2). The O-antigens are the major antigenic factors responsible for the serological properties. More than 50 serotypes have by now been distinguished among *Y. enterocolitica* and closely related species (Fredriksson-Ahomaa 2001; Hudson et al. 2008; Virdi and Sachdeva 2005).

Table 1 Biotyping scheme for *Y. enterocolitica* (modified from Bottone 1999)

Biochemical reaction	Reactions in different *Y. enterocolitica* biotypes					
	1A	IB	2	3	4	5
Inositol fermentation	+	+	+	+	+	+
Salicin fermentation	+	–	–	–	–	–
Sorbose fermentation	+	+	+	+	+	–
Trehalose fermentation	+	+	+	+	+	–
Ornithine decarboxylase activity	+	+	+	+	+	+
Lipase activity	+	+	–	–	–	–
Pyrazinamidase activity	+	–	–	–	–	–
Nitrate reduction	+	+	+	+	+	–
Esculin hydrolysis	+/–	–	–	–	–	–

+, positive result (activity present); **–**, negative result (activity absent); **+/–**, present or absent (depends on the examined bacterial strain)

Table 2 Biotype association of virulent *Y. enterocolitica* serotypes (modified from Bottone 1999)

Bio type	Serotype(s)
1B	O:8; O:4; O:13a,13b: O:18; O:20; O:21
2	O:9; O:5,27
3	O:1,2,3; O:5,27
4	O:3
5	O:2,3

Cell Surface Structures in *Y. enterocolitica*

Architecture of the microbial cell wall (CW) significantly differs among bacteria, depending strictly on their affiliation to the Gram-negative or Gram-positive group (Fig. 1). While in Gram-negative bacteria CW consists of three major layers: the cytoplasmic or inner membrane (IM), peptidoglycan-impecunious periplasmic space and the external outer membrane (OM), in Gram-positive bacteria CW is much thicker and built of the IM and 3-dimensional peptidoglycan network. Various surface-exposed structures present in the OM outer leaflet play a crucial role in the survival of all known Gram-negative bacteria. In virulent bacteria, the cell surface components have a dual role both as virulence factors that interact with a variety of eukaryotic receptors and as factors maintaining the proper architecture of the OM.

LPS: the Multifaceted Surface Component

Lipopolysaccharide, also known as endotoxin, is a major component of the outer leaflet of bacterial OM (Silhavy et al. 2010). For most Gram-negative bacteria, LPS is crucial for the survival, however, the recent discovery of the LPS-lacking *Sorangium cellulosum* proves that bacteria may survive without LPS (Keck et al. 2011).

LPS was discovered by the German physician and bacteriologist Richard Pfeiffer in 1892, during his studies on the virulence of *Vibrio cholerae*. He demonstrated that LPS was responsible for the toxicity of the heat-killed *V. cholerae* cells (Beutler and Rietschel 2003; Lüderitz et al. 1982; Wang and Quinn 2010).

LPS consists of two physicochemically distinct moieties, the hydrophobic lipid and the hydrophilic polysaccharide parts. The lipid part is responsible for the toxicity of LPS while the serological properties of LPS are due to the polysaccharide (Erridge et al. 2002; Seltmann

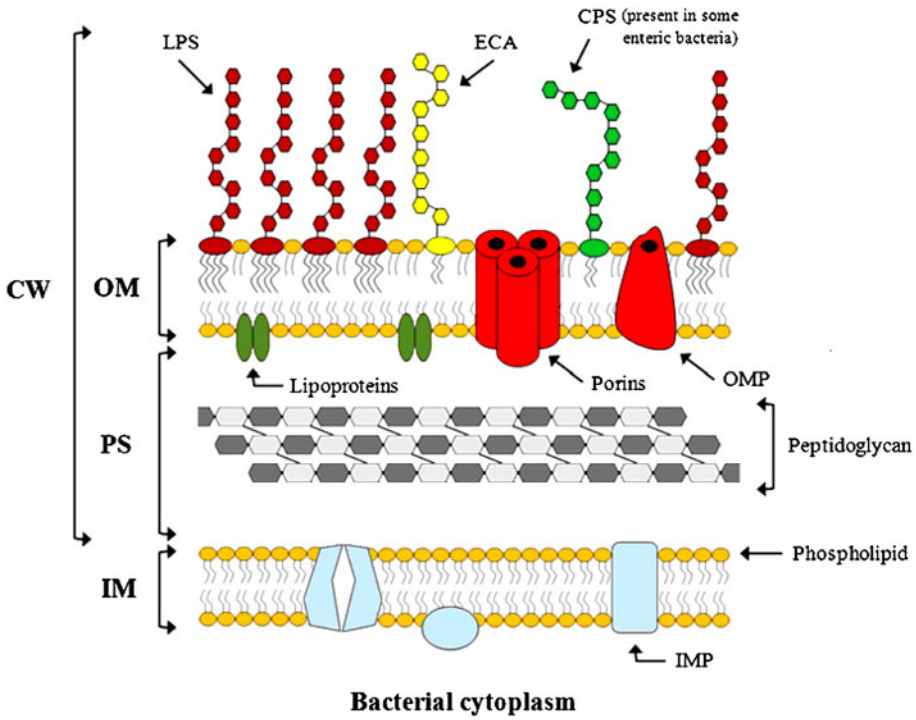

Fig. 1 Molecular architecture of the enterobacterial cell wall (*CW*) (modified from Alexander and Rietschel 2001). *IM* inner membrane, *IMP* inner membrane protein, *PS* periplasmic space, *OM* outer membrane, *OMP* outer membrane protein, *CPS* capsular polysaccharide, *ECA* enterobacterial common antigen, *LPS* lipopolysaccharide

and Holst 2002). Moreover, in the composition of a single LPS molecule, three diverse parts can be distinguished: lipid A that is anchored in the OM, the core oligosaccharide with its internal (inner core: IC) and external (outer core: OC) parts and the O-PS or O-antigen that is exposed to cell surrounding. The three basic LPS forms are called smooth (S), semi-rough (SR) and rough (R). Smooth LPS is present in most wild-type *Enterobacteriaceae* species and it contains all three LPS parts. The SR and R LPS forms are most easily observed in respective LPS mutants that express an incomplete polysaccharide part. In SR-mutants due to a missing O-unit polymerase activity LPS carries only one O-repeating unit and in R-mutants the O-PS is completely missing. Furthermore, among R-mutants, several LPS chemotypes are recognized based on the observed differences in the core structures. Ra-mutants possess a complete core, Rc-mutants are missing the OC. LPS of Re-mutants consists of only lipid A substituted with Kdo residues (2-keto-3-deoxy-D-*manno*-octulosonic acid) (Bruneteau and Minka 2003; Duda et al. 2009; Kenne and Lindberg 1983; Knirel and Kochetkov 1994; Lüderitz et al. 1982; Rietschel et al. 1991; Seltmann and Holst 2002).

The most conservative component of the bacterial LPS is lipid A that is made of two glucosamine (GlcN) residues linked together via a β-1,6-glycosidic bond. The 2, 3, 2′ and 3′ hydroxyl groups of the biphosphorylated backbone GlcN–GlcN disaccharide are substituted with saturated fatty acids laurate (C12:0) or myristate (C14:0). The hydrophobic and hydrophilic LPS parts are coupled via an acid-labile ketosidic bond between the 6′-carbon of lipid A GlcN and the proximal Kdo molecule (Aussel et al. 2000; Bruneteau and Minka 2003; Karabian et al. 1999; Oertelt et al. 2001; Seltmann and Holst 2002). Interestingly in *Y. enterocolitica*, the level of lipid A acylation is dependent on the growth temperature. Bacteria grown at 21 °C have a hexa-acylated lipid A, while at 37 °C, it is preferentially tetra-acylated. It is speculated that reduction of the lipid A hydrophobicity would increase the OM fluidity and permeability that in turn would enhance the acquisition, transport and exchange of lipophilic nutrient substances in pathogenic *Y. enterocolitica* within host tissues. In addition, due to reduced endotoxicity, it might be more difficult for the host innate immune response mechanisms to identify the hypo-acylated lipid A structure, and thereby, the presence of the enteropathogenic bacteria (Bengoechea et al. 2003; Medzhitov and Janeway 2000; Perez-Gutierrez et al. 2010; Seydel et al. 1999).

Bioreactivity of lipid A is determined by its phosphate substituents and fatty acid chains which are crucial in the activation of Toll-like receptor 4 and CD14 receptors in host macrophages (Loppnow et al. 1989; Persing et al. 2002; Rietschel et al. 1994). The overall negative charge of lipid A gives the bacteria a possibility to bind and immobilize cationic antimicrobial peptides (CAMPs) (Wang and Quinn 2010).

The core oligosaccharide is also an important part of LPS. The IC consists of the Kdo and heptose residues while the OC generally contains different hexoses (Seltmann and Holst 2002). In the core of *Y. enterocolitica* LPS, the following sugar residues are usually present: glucopyranose

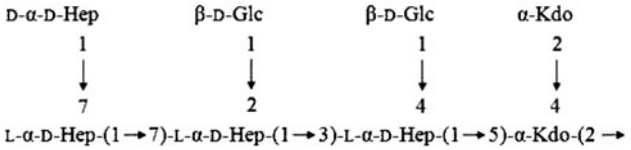

Fig. 2 Schematic structure of the LPS IC in serotypes O:3, O:8 and O:9 of *Y. enterocolitica* (Müller-Loennies et al. 1999; Oertelt et al. 2001; Pinta et al. 2010; Radziejewska-Lebrecht et al. 1994). D-Glc, D-glucose; D,D-Hep, D-*glycero*-D-*manno*-heptose; L,D-Hep, L-*glycero*-D-*manno*-heptose; IC, inner core; Kdo, 2-keto-3-deoxy-D-*manno*-octulosonic acid

(Glc*p*), galactopyranose (Gal*p*), 2-acetamido-2-deoxy-D-galactopyranose (Gal*p*NAc), L-*glycero*-D-*manno*-heptose (L,D-Hep), D-*glycero*-D-*manno*-heptose (D,D-Hep) and Kdo (Acker and Wartenberg 1976; Bruneteau and Minka 2003; Holst 2007; Radziejewska-Lebrecht et al. 1994). The IC structure of *Y. enterocolitica* has a common backbone of D,D-Hep-(1→7)-L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-[Kdo-(2→4)]-Kdo (Fig. 2) (Bruneteau and Minka 2003; Oertelt et al. 2001; Radziejewska-Lebrecht et al. 1994). In *Y. enterocolitica*, O:3 LPS a rarely encountered 4-keto-hexosamine (2-acetamido-2,6-dideoxy-D-*xylo*-hex-4-ulopyranose or Sug*p*) acts as a linker between the OC and the IC (Holst 2007; Pinta et al. 2009). The Sug*p* mislead successfully the chemists for years. Due to its sensitivity to hydrazine treatment it appeared as either *N*-acetyl-D-fucosamine (FucNAc) or *N*-acetyl-D-quinovosamine (QuiNAc) in the analyses. The OC hexasaccharide of *Y. enterocolitica* O:3 (YeO3) in addition to the proximal Sug*p* residue contains two Glc, one Gal and two GalNAc residues (Pinta et al. 2009; Radziejewska-Lebrecht et al. 1998).

The *Y. enterocolitica* O:3 OC gene cluster contains in addition to six genes encoding glycosyltransferases (GTases) required in the formation of the OC, the *wzx*, *wbcP* and *gne* genes. While the Wzx protein acts as a flippase responsible for translocation of undecaprenyl phosphate (Und-P) linked OC hexasaccharide through the IM, WbcP is UDP-*N*-acetylglucosamine-4,6-dehydratase that converts UDP-Glc*p*NAc to UDP-Sug*p*, and Gne is UDP-*N*-acetylglucosamine-4-epimerase that converts UDP-Glc*p*NAc to UDP-Gal*p*NAc (Bengoechea et al. 2002; Pinta et al. 2010).

The six GTases involved in the biosynthesis of YeO3 OC are WbcO, WbcQ, WbcN, WbcM, WbcL and WbcK. These enzymes are responsible for the sequential transfer of hexose residues onto the lipid carrier Und-P. The biosynthesis of YeO3 OC is initiated with the transfer of Sug*p*-P from UDP-Sug*p* onto Und-P, a reaction that is catalyzed by WbcO, the priming GTase (UDP-Sug*p*:Und-P transferase). WbcQ is the UDP-GalNAc:Und-P–P-Sug*p*-α-(1→3)-*N*-acetylgalactosaminyltransferase that forms the α-(1→3)-linkage between GalNAc (I) and Sug*p*. WbcN is the galactosyltransferase forming the Gal-α-(1→4)-GalNAc (I) linkage, and WbcM transfers the GalNAc (II) and forms the α-(1→6)-linkage to the Gal residue. WbcL and WbcK are glucosyltransferases that both need a specific acceptor to act as a transferase. WbcL is the Glc (I) transferase that forms the β-(1→3)-linkage to GalNAc (I) but it cannot transfer the Glc (I) residue before the GalNAc (II) has been added to the growing oligosaccharide. WbcK is the Glc (II) transferase forming the β-(1→6)-linkage to GalNAc (II) but only after Glc (I) residue has been transferred by WbcL (for YeO3 OC composition see Fig. 3) (Pinta et al. 2010).

The LPS core of many Gram-negative pathogens (especially its external part) functions as a bacteriophage receptor, activates the host complement system, interacts with serum proteins other than antibodies (especially the IC), provides resistance to CAMPs and determines serological properties of SR- and R-mutants (Erridge et al. 2002; Pinta et al. 2009).

The O-PS is the third major component of LPS. The antigenic identity of the O-PS is determined by its sugar composition, by the anomeric configuration and the optical rotation of its building units and finally by the nature of the *O*-glycosidic bonds present in its molecular structure (Bruneteau and Minka 2003; Gorshkova et al. 1985; Seltmann and Holst 2002). In wild-type *Y. enterocolitica* O:3 and O:9 (YeO9) strains, the O-PS are linear homopolysaccharides made of 6-deoxy-L-altropyranose (6d-Alt*p*) and *N*-formylperosamine (Rha*p*4NFo), respectively. Unlike in other *Enterobacteriaceae*, the O-PS in *Y. enterocolitica* O:3 is not linked to the LPS OC (Fig. 3) (Skurnik et al. 1995). The O-PS of *Y. enterocolitica* O:8 (YeO8) is a branched heteropolysaccharide containing residues of Gal*p*NAc, Gal*p*, L-fucopyranose, D-mannopyranose, and 6-deoxy-D-gulopyranose (Fig. 4) (Bruneteau and Minka 2003; Caroff et al. 1984; Hoffman et al. 1980; Tomshich et al. 1987).

The O-specific polysaccharide has many biological roles as it functions as a bacteriophage receptor, enhances the invasive and colonizing abilities of adhesive factors of certain enteric bacteria, and plays a significant role in determining microbial resistance to host complement system (Biedzka-Sarek et al. 2008; Erridge et al. 2002; Lüderitz et al. 1982).

In *Y. enterocolitica* O:3 expression of the O-PS and OC is temperature-regulated and most efficient in temperatures lower than 30 °C (Biedzka-Sarek et al. 2008). At room temperature, wild-type YeO3 strains exhibit more abundant O-PS than at 37 °C (Acker et al. 1981; al-Hendy et al. 1991; Kawaoka et al. 1983; Wartenberg et al. 1983).

In general, the endotoxin acts as a forceful activator of the host innate immune system. It stimulates the leukocyte

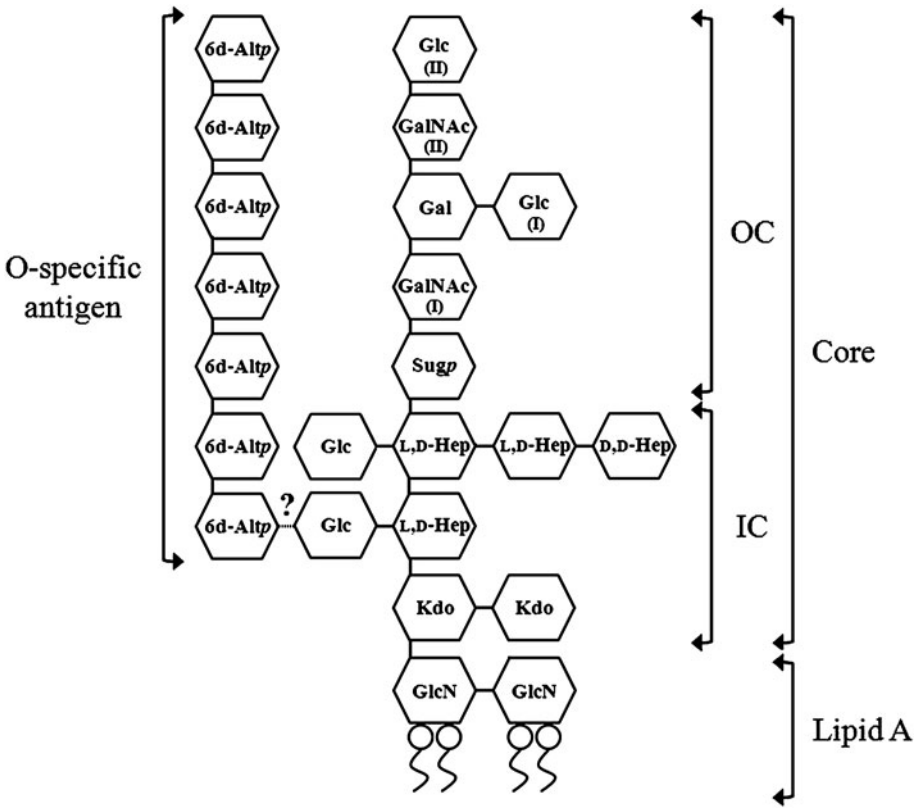

Fig. 3 Schematic structure of *Y. enterocolitica* O:3 complete lipopolysaccharide (Ye75S) (modified from Holst 2007; Pinta et al. 2009; Skurnik et al. 1995). 6d-Alt*p*, 6-deoxy-L-altropyranose; Gal, galactose; GalNAc, *N*-acetyl-D-galactosamine; Glc, glucose; GlcN, glucosamine; D,D-Hep, D-*glycero*-D-*manno*-heptose; L,D-Hep, L-*glycero*-D-*manno*-heptose; IC, inner core; Kdo, 2-keto-3-deoxy-D-*manno*-octulosonic acid; OC, outer core; Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hex-4-ulopyranose; "**?**", a hypothetical location of the *O*-glycosidic bond linking O-PS to IC, however, there is no experimental evidence for that

inflammatory cytokine output. The toxicity of LPS in host depends on the amount in which it is present in the system. Small concentrations of endotoxin molecules might be beneficial in overcoming some bacterial infections. They stimulate the immune mechanisms and this induces antibacterial, antiviral and antitumor immunity in host. Higher concentrations of LPS are already deleterious. Beside pathological conditions of the circulatory system, e.g., hypertension, agranulocytopenia, disseminated intravascular coagulation and hypoferremia, the life-threatening septic shock or multiple organ dysfunction might be the results of a cytokine boost in host. Bioreactivity of LPS is impressive as it does not only induce the host immunity mechanisms but also widely affect the basic cellular metabolism (Parrillo 1993; Swain et al. 2008; Wang and Quinn 2010).

Serotype	Structure of the O-PS
O:3	→ 2)-β-L-6dAlt*p*-(1 →
O:8	6d-D-Gul*p* L-Fuc*p* 1 1 ↓ ↓ 3 2 → 4-D-Man*p*-(1→3)-D-Gal*p*-(1→3)-α-D-GalNAc*p*-(1 →
O:9	→ 2)-α-D-Rha*p*4NFo-(1 →

Fig. 4 The O-PS repeat unit structures of *Y. enterocolitica* serotypes O:3, O:8 and O:9 (Caroff et al. 1984; Hoffman et al. 1980; Tomshich et al. 1987). L-6dAlt*p*, 6-deoxy-L-altropyranose; L-Fuc*p*, L-fucopyranose; D-Gal*p*, D-galactopyranose; D-GalNAc*p*, *N*-acetyl-D-galactosaminopyranose; 6d-D-Gul*p*, 6-deoxy-D-gulopyranose; D-Man*p*, D-mannopyranose; D-Rha*p*4NFo, 4,6-dideoxy-4-formamido-D-mannopyranose

In bacteria, virulence is not dependent on the form of possessed LPS. It has been evidenced that even bacteria that originally synthesize a truncated LPS (lacking the O-PS or both the O-PS and the core oligosaccharide) might be highly infectious, e.g., *Francisella tularensis* (Wang et al. 2006) and *Y. pestis* (Seltmann and Holst 2002). In some enteric bacteria, the loss of O-PS leads to a significant decrease in the inborn virulence (al-Hendy et al. 1992; Chart et al. 1989).

The complete LPS in pathogenic *Y. enterocolitica* is necessary for the bacteria to retain full virulence. A comparison of the pathogenicity of smooth YeO3 and YeO8 strains and their rough mutants demonstrated that the O-PS lacking bacteria were, respectively, 50- and 100-fold less virulent than the wild-type strains (al-Hendy et al. 1992; Zhang et al. 1997). Moreover, it has also been reported that in YeO3, the OC is an important virulence factor (Skurnik et al. 1999).

Enterobacterial Common Antigen: the Familial "Tattoo" of *Enterobacteriaceae*

The chromosome-encoded cell surface glycolipid enterobacterial common antigen (ECA) was discovered by Kunin (1962), when he was examining cross-reactions of indirect hemagglutination between various *Escherichia coli* O-serotypes. ECA has been found in almost all *Enterobacteriaceae*. ECA is a significant component of the outer leaflet of OM. The presence of ECA in *Yersinia* was a key reason to reclassify *Yersinia* from *Pasteurellaceae* to *Enterobacteriaceae* (Bercovier and Mollaret 1984; Gromska and Izdebska-Szymona 1987; Radziejewska-Lebrecht et al. 1998; Seltmann and Holst 2002).

Three forms of ECA have been found in enteric bacteria. They all share a common structure of the trisaccharide repeat units but differ in the presence or absence of the lipid moiety and in the localization in the bacterial cell. ECA_{PG} is present in most *Enterobacteriaceae* and the polysaccharide is linked to phosphoglyceride aglycone. In ECA_{LPS}, the ECA carbohydrate chain is covalently ligated to the core-lipid A. ECA_{PG} and ECA_{LPS} are localized in the outer leaflet of the OM. ECA_{CYC}, unlike the ECA_{PG} and ECA_{LPS}, is assembled in the periplasmic space into a lipid-free cyclic polysaccharide. While the hydrophilic part of ECA is responsible for the serological properties of ECA, the hydrophobic moiety determines the micelle-forming ability and wide range of adhesion capabilities of ECA (Duda et al. 2009; Kajimura et al. 2005; Kuhn et al. 1988).

ECA_{LPS} and ECA_{PG} differ based on their immunogenicity. ECA_{LPS} is immunogenic and induces the host immune response to produce specific anti-ECA antibodies while the non-immunogenic ECA_{PG}, also known as haptenic ECA, does not stimulate antibody production unless conjugated to a protein carrier (Kuhn et al. 1988; Seltmann and Holst 2002; Whang et al. 1972).

The repeat unit of ECA contains three aminosugars: 4-acetamido-4,6-dideoxy-D-galactose (Fuc4NAc), *N*-acetyl-D-mannosaminuronic acid (ManNAcA) and *N*-acetyl-D-glucosamine (GlcNAc). They form a trisaccharide repeating unit with the chemical formula of [→3)-α-Fuc4NAc-(1→4)-β-ManNAcA-(1→4)-α-GlcNAc-(1→] that is polymerized to ECA-polysaccharide of different chain lengths to form the hydrophilic moiety of ECA. The biochemical modifications of ECA have received increasing interest as they may influence the virulence properties of the enteric pathogens (Kajimura et al. 2006).

We have studied the presence and expression of ECA in *Y. enterocolitica* O:3 strains. The presence of the immunogenic ECA_{LPS} was observed both in wild-type smooth bacteria and in different R-mutants. The wild-type strain Ye75S turned out to express ECA_{LPS} along with complete O-chain-bearing LPS which affects the serological properties of the bacteria (Duda et al. 2009; Kasperkiewicz et al. 2004; Radziejewska-Lebrecht et al. 1998, 2003). The presence of ECA_{LPS} in bacteria seems not to be incidental since the biosynthetic pathways of ECA and LPS share many cellular components such as similar or identical carbohydrate precursors and the Und-P as well as some glycosyltransferases (Kuhn et al. 1988; Seltmann and Holst 2002). Using immune-electron microscopy, it was demonstrated that cultivation temperature of Ye75S, the wild type strain of *Y. enterocolitica* O:3 significantly influenced the level of ECA exposition on bacterial cell surface. The anti-ECA antibodies did not bind to bacteria grown at 22 °C, because ECA was covered by abundant O-PS while this was not the case in bacteria grown at 40 °C (Acker 1977, 1981; Hoffman et al. 1980). It has been recently discovered that the ECA expression is temperature-regulated in YeO3 and more efficient at 22 °C and decreased at 37 °C (Rabsztyn et al. 2011).

The biological role of ECA in *Enterobacteriaceae* is not fully known. In a few virulence experiments performed thus far, the ECA-negative bacteria demonstrated some reduction of virulence when compared to isogenic ECA-positive bacteria (Valtonen et al. 1976). Furthermore, patients suffering from enterobacterial infections such as: rhinoscleroma (Kałużewski 1974), chronic urinary tract infections, Leśniowski–Crohn's disease, purulent peritonitis, ulcerative colitis and reactive arthritis have increased levels of anti-ECA antibodies in sera (Aoki et al. 1996; Bartnik and Kałużewski 1979; Diaz and Neter 1968; Malkamäki 1981). However, ECA does not exhibit either endotoxic or cytotoxic activities (Mayer and Schmidt 1979).

Myf Antigen: the Misleading Envelope-Like Structure in Some *Y. enterocolitica*

Presence of the mucoid *Yersinia* factor (Myf antigen) in *Y. enterocolitica* was discovered when investigating the morphology and specific biochemical properties of the colonies cultivated at 37 °C under acidic conditions (Diaz et al. 1985). Biosynthesis of the Myf antigen is determined by the transcription of the chromosomal *myf* locus containing five genes, i.e., *myfA*, *myfB*, *myfC*, *myfE* and *myfF*. The first sequence encodes the MyfA protein, the basic component of the Myf antigen. Expression of the next two genes results in the synthesis of specific periplasmic and OM proteins responsible for the proper architecture of the Myf antigen. Genes *myfE* and *myfF* encode IM proteins that act as regulators of the *myfA* transcription at 37 °C (Iriarte and Cornelis 1995).

The major subunit MyfA is a 21-kDa polypeptide of 159 amino acids (aa). Interestingly, one-third of MyfA (approximately 51 aa) comprises its N-terminal signal

sequence that is cleaved off by a protease when MyfA is translocated from the bacterial cytoplasm to the OM. Therefore, the size of a mature MyfA subunit is about 14-kDa that polymerize to form the Myf antigen, a fibrous layer that covers the bacteria and results in the characteristic colony morphology (Diaz et al. 1985; Iriarte et al. 1993).

Although the precise function of the Myf antigen in *Y. enterocolitica* is still unknown, virulence experiments suggest that Myf may interact with the host intestinal epithelium. Moreover, Myf may protect *Y. enterocolitica* from the bactericidal activity of host phagocytes (Diaz et al. 1985).

Inv: the Molecular Leader of Host Intestinal Infection by *Y. enterocolitica*

The chromosomally encoded Inv (92-kDa) is a 987 aa polypeptide. Invasin (Inv) synthesis in *Y. enterocolitica* is growth phase-dependent. It is maximally expressed when bacteria transit from logarithmic to stationary phase of growth at both 28 and 37 °C, in the higher temperature under acidic conditions (Bottone 1999; Pepe et al. 1994). The biosynthesis of invasin in vivo has been evidenced in *Y. enterocolitica* (Pepe and Miller 1993). While in *Y. enterocolitica* serotypes O:8 and O:9, Inv is expressed efficiently at environmental temperatures and poorly at 37 °C, in serotype O:3 production of invasin is constitutive both at moderate as well as at higher temperatures (Uliczka et al. 2011). The authors found out that the reason for the difference in regulation was due to an IS1667-element insertion into the *invA* promoter region in YeO3. In addition, the activator RovA carries a substitution that renders the protein more resistant to proteolysis. These differences resulted in strong Inv-expression both at 22 and 37 °C, however, YeO3 is invasive only at 37 °C when O-PS expression is repressed exposing Inv (Uliczka et al. 2011). At the same time, there is evidence that invasin and motility are co-regulated with a reverse correlation between synthesis of invasin and flagella, as environmental stimuli and regulatory proteins that enhance one of the processes will simultaneously inhibit the second one. When *Y. enterocolitica* adheres to host gut wall, there is no longer a need for the bacterium to stay motile (Badger and Miller 1998). Moreover, it is believed that RovA may act as a pleiotropic regulator and beside the expression of the *invA* gene it could regulate transcription of genes encoding other virulence factors of *Y. enterocolitica*. This hypothesis is based on the virulence experiments where *Y. enterocolitica rovA* mutants were less virulent than the *invA* mutant and wild-type counterparts (Dube et al. 2003; Marceau 2005; Revell and Miller 2000).

Invasin is a cell surface adhesin directly engaged in the first phase of infection and initiates the internalization of the bacteria to small intestine epithelial cells, especially to M cells, present in its distal section of ileum (Isberg and Leong 1990; Jepson and Clark 1998). M cells cover the Peyer's patches, the intestinal mucosal lymphoid nodules that are an important part of the gut-associated lymphoid tissue. Penetration of the M cells by *Y. enterocolitica* begins with a direct interaction between Inv molecules and any of the five types of β_1 integrins ($\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_1$) on the surface of target epithelial cells. Inv stimulates a specific remodeling of actin filaments in the M cell cytoskeleton and the bacteria are invaginated into the cells ending up within endosomal vesicles. Moreover, it has been discovered that Inv plays an important role in inducing autophagocytosis in macrophages (Deuretzbacher et al. 2009). The bacteria pass through the M cells and are released into the lamina propria where the bacteria reside mainly extracellularly (Autenrieth and Firsching 1996).

YadA: a Collagen Binding Adhesin and the Major *Y. enterocolitica* Weapon against Host Complement System

The y*adA* gene is located on the *Yersinia* virulence plasmid (pYV) and its expression is mainly temperature-regulated occurring at 37 °C. The two regulators of the *yadA* gene transcription are VirF (plasmid-borne transcriptional activator of *yadA*) and YmoA (chromosome encoded transcriptional repressor of *virF*). The YmoA protein, a histone-like inhibitor, prevents expression of VirF at temperatures below 37 °C. The latter, belonging to a family of AraC transcription regulators, is responsible for YadA synthesis exclusively at 37 °C. Beside *yadA* the genes encoding several other *Y. enterocolitica* virulence factors, e.g., *Yersinia* outer proteins are under regulation of the VirF activator. It is believed that in response to temperature, bacteria modify the genome structure rendering some promoters better accessible to transcription activators (Lambert de Rouvroit et al. 1992). Other extracellular factors efficiently inducing the transcription of *yadA* gene are *inter alia*: pH, osmolarity, concentration of Fe^{3+} ions, adequate availability of nutrient substances and the direct interaction between the pYV-bearing bacterium and host eukaryotic cells, especially its phagocytes (Bölin et al. 1985; Portnoy et al. 1984).

The YadA molecule is exposed about 30 nm to the surrounding of *Y. enterocolitica* (Koretke et al. 2006). In the structure of the lollipop-shaped YadA adhesin, three major regions can be easily distinguished, i.e., a 5-nm long N-terminal globular head domain, an 18-nm long coiled-coil helical stalk and a short C-terminal OM anchor (Hoiczyk et al. 2000; Journet et al. 2003; Nummelin et al. 2004; Skurnik et al. 1984; Skurnik and Wolf-Watz 1989; Zaleska et al. 1985). YadA is an archetype of a family of

oligomeric coiled-coil adhesins (Oca) common in Gram-negative pathogens (Hoiczyk et al. 2000). These extracellular proteins are either Vc-type or trimeric autotransporters such as YadA (Cotter et al. 2005; Henderson et al. 2004). The basic difference between them is that head domains of the latter, instead of being cleaved off, act as integral components of these oligomeric proteins at bacterial cell surfaces (Cotter et al. 2005). A characteristic feature of the Oca family members is that approximately 70 of their last C-terminal amino acid residues form a 12-stranded β-barrel pore within the bacterial OM (Ackermann et al. 2008). YadA head domain is responsible for autoagglutination, binding to both neutrophilic granulocytes and host extracellular matrix components such as collagen, laminin and fibronectin (Schulze-Koops et al. 1992, 1993). Due to this it facilitates adhesion of *Y. enterocolitica* to host ileocecal epithelium (Bottone 1997; El Tahir and Skurnik 2001; Hoiczyk et al. 2000; Lachica and Zink 1984; Skurnik et al. 1984; Skurnik and Wolf-Watz 1989; Zaleska et al. 1985). The stalk is responsible not only for extending the head domain over the bacterial surface but also for complement resistance. The OM anchor is responsible for the translocation and oligomerization of the trimeric polypeptide through the membrane and finally anchoring YadA to OM (Biedzka-Sarek et al. 2008; Hoiczyk et al. 2000; Roggenkamp et al. 2003). YadA binds the host serum factor H and C4b binding protein that act as negative regulators of the alternative and classical pathways of complement activation, respectively (Kirjavainen et al. 2008). Using these molecules, the bacteria efficiently perturb the complement activating cascade preventing C3b deposition and MAC formation and efficiently promote the survival of *Y. enterocolitica* in host tissues. YadA cooperates in complement resistance with the Ail protein (Biedzka-Sarek et al. 2008).

Ail: the Poorly Exposed Co-Creator of *Y. enterocolitica* Serum Resistance

The attachment-invasion protein (Ail) is a 17-kDa *Y. enterocolitica* cell surface adhesin, whose biosynthesis, observed exclusively at 37 °C, is affected by pH and oxygen content (Pederson and Pierson 1995). Due to its small size, Ail is easily covered by other surface structures on *Y. enterocolitica* cells. The 178 aa Ail polypeptide folds into eight amphipathic β-sheets in the OM and four short loops are exposed to the cell surroundings. They are regarded as major determinants of the biological functions of Ail and also responsible for binding of factor H. Cooperation between YadA and Ail ensures a high level of serum resistance (Bliska and Falkow 1992; Bottone 1997; Miller et al. 2001). Interestingly, due to the small size of Ail, to efficiently bind factor H, Ail needs to be fully exposed on bacterial cell surface and not blocked by the distal parts of LPS such as O-PS or OC (Biedzka-Sarek et al. 2008).

Conclusions

The natural environment is a great reservoir of virulent and avirulent bacterial species. Virulent bacteria are parasites that exploit either other prokaryotes or eukaryotes. Precise bacterium-host cell interactions are crucial for the successful pathogenesis in all microbial infections, and in most cases, this depends on specific bacterial cell surface structures. For *Y. enterocolitica* some of the structures have been well characterized while others are still the subject of many investigations. Comprehensive knowledge of the chemical composition, structural architecture, physico-chemical properties and regulatory/co-regulatory pathways of *Y. enterocolitica* surface components is necessary to help us in understanding the molecular details of the disease process, and perhaps in future, in producing novel antimicrobials that would specifically block the surface components.

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