Bacterial Polysaccharides: An Overview

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Contents

1 Introduction 82
2 Peptidoglycan 84
3 Teichoic Acids 87
4 Bacterial Glycoproteins 91
5 Membrane-Derived Oligosaccharides 94
6 Lipopolysaccharides 95
7 Capsular Polysaccharide 99
8 Future Outlook 103
References 104

Abstract

Bacterial cell wall and membrane are associated with a variety of glycoconjugates and polysaccharides which aids in structural formation as well as performing various functions in the bacterial cell. In gram-negative bacteria, peptidoglycan is majorly present in the periplasmic space and it provides mechanical strength as well as shape to the cell. In some cases, the periplasm contains membrane-derived oligosaccharides (MDOs), which are involved in osmoregulation. The outer membrane mainly contains lipopolysaccharides (LPSs) that bind to divalent cations or chelators for structure stabilization and to increase outer membrane permeability. This LPS contains lipid A, also known as endotoxin, which has shown a powerful biological effect in mammals such as fever, septic shock, multiple organ failure, and mortality. The mucoid (slime-producing) strains contain capsular polysaccharide which

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aids as virulence factor. The gram-positive bacteria lack an outer membrane and have a much thicker peptidoglycan layer along with a specialized polysaccharide known as teichoic acid. It provides cell wall integrity through complex formation with cations and also assists in cell growth regulation. The present report attempts to provide an overview of bacterial polysaccharide structure, occurrence, and their important functions, along with the biosynthesis and major inhibitors to block biosynthetic pathways.

Keywords

Peptidoglycan • Teichoic acid • Bacterial glycoproteins • Membrane-derived oligosaccharides • Lipopolysaccharides • Capsular polysaccharide

Abbreviations

ABC	ATP-binding cassette
ACP	Acyl carrier protein
CPS	Capsular polysaccharide
Gal	Galactose
Glc	Glucose
GlcNAc	*N*-Acetylglucosamine
GT	Glycosyltransferase
HMM-PBPs	High molecular mass PBPs
LAL assay	Limulus amebocyte lysate assay
LOS	Lipooligosaccharides
LPS	Lipopolysaccharides
LTA polymers	Lipoteichoic acid polymers
LDAP	L-Diaminopimelic acid
Man	Mannose
MDOs	Membrane-derived oligosaccharides
MurNaC	*N*-Acetylmuramic acid
NBD	Nucleotide-binding domain
OPGs	Osmoregulated periplasmic glucans
PBPs	Penicillin-binding proteins
PG	Lysophosphatidylglycerol
WTAs	Wall TAs

1 Introduction

In nature, mostly the carbohydrates exist as polymers rather than monomers. In this context, polysaccharides are believed to be the most abundant polymeric carbohydrate. These polymers consist of ten or more simple sugar units and composed of thousands or hundreds of thousands of simple sugars which are bonded together by glycosidic bonds so as to have molecular masses as high as 100 million atomic mass units. The main function of the polysaccharide is determined by the monomers it

contains or the manner in which they are linked together. Polysaccharides present in plants, animals, and bacteria are majorly responsible for their structural and functional roles. The most common polysaccharides are cellulose in plants and glycogen in animals. They act as storage polysaccharides while certain others provide the structural support and are known as structural polysaccharides. Among the structural polysaccharides is chitin which is generally present in invertebrate animals.

On the basis of morphological localization, bacterial polysaccharides could be divided into groups: intracellular polysaccharides which are located inside or as part of the cytoplasmic membrane or cell wall such as peptidoglycan, periplasmic glucans, lipopolysaccharide (LPS), lipooligosaccharide (LOS), and capsular polysaccharides which form a structural part of the cell wall (Chawla et al. 2009) or the extracellular polysaccharides also known as exopolysaccharides consisting of branched, repeating units of sugars or sugar derivatives. Extracellular polysaccharides are further classified as: homopolysaccharides (cellulose, dextran, pullulan, curdlan) and heteropolysaccharides (gellan, xanthan) on the basis of the sugar or its derivative a particular molecule contains. Bacteria and many other microbes including fungi and algae often secrete diverse polysaccharides as an evolutionary adaptation to help them adhere to the surface as part of the cell wall component or storage units or virulence factors and also to prevent them from drying out. Exopolysaccharides have shown wide industrial applications mainly in food and pharmaceutical products and to certain extent in textile, paper, and cosmetics, as gelling agents and medicines for wound dressings (Sutherland 1998). In context to exopolysaccharides, accumulation of polyhydroxybutyrate (PHB) has been extensively studied. This is a kind of polyesters which is synthesized by a number of microorganisms as energy reserve materials under unfavorable conditions, i.e., in the limitation of some essential nutrients under an excess availability of carbon source (Lee 1996; Khanna and Srivastava 2005). The properties of pure PHB are comparable to the commonly used petroleum-derived bulk plastics, e.g., polypropylene. It is a unique natural biopolymer, which exhibits three exceptional features (Hrabak 1992): (i) thermoplastic process ability, (ii) 100 % resistance to water and moisture, and (iii) 100 % biodegradability. It could therefore be used for applications similar to those of common plastics and would fit well into new waste disposal and management strategies. While working with PHB production, it was observed that the fermented broth containing 125 gl^{-1} of sucrose had resulted in 22.65 gl^{-1} of PHB from 29.7 gl^{-1} of dry cell weight (DCW) in 38 h at 33 °C, 200 rpm by *A. australica* using constant feed rate (100 ml/h for 15 h) in fed-batch cultivation (Gahlawat and Srivastava 2013). Recently, an attempt has been made on the usage of renewable resources such as glycerol (biodiesel by-product) in order to make the process sustainable and cost effective and ensure renewabilities. The microorganisms investigated in the present study using these categories of substrates are *Alcaligenes latus*, *Ralstonia eutropha*, and *Bacillus* sp. (data not shown). The biopolymer produced by *Bacillus* sp. will be suitable for medical use, and to develop an efficient production process, studies are continuing in author's laboratory.

In the light of the current state of the art on bacterial intracellular polysaccharides, the bacterial cell wall deserves special attention as it is important for maintaining the cell viability by protecting the cell protoplast from mechanical damage either through osmotic rupture or lysis. The bacterial cell wall is composed of various unique components which are not present in nature; it is also believed to be the most important site for the attack by antibiotics. The cell wall provides ligands for the adherence and also the receptor sites for drugs or viruses. They provide immunological distinction among the bacterial strains (Todar 2011).

Realizing the important features of bacterial cell wall, this communication attempts to elucidate varied bacterial intracellular polysaccharide characteristics which are generally present in the cell wall or in the cellular membrane, their structure, occurrence, importance, or the functional role played in the cell, and its applications for human welfare and future prospects.

2 Peptidoglycan

Peptidoglycan (murein) is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of bacteria (except Archaea), which forms the cell wall. The peptidoglycan in most of the gram-negative bacteria consists of alternating residues of β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNaC). *N*-acetylmuramic acid is attached covalently to short tetrapeptide chains which are composed of L-Ala, unusual D-amino acids (D-Glu and D-Ala), and L-diaminopimelic acid (LDAP). These unusual D-amino acids do not generally occur in proteins and therefore help to protect against the attacks by most peptidases. The tetrapeptide side chains could also be linked to one another by an interpeptide bond between a free amino group on diaminopimelic acid (DAP) of one chain and free carboxy group on nearby D-Ala on the other tetrapeptide side chain of *N*-acetylmuramic acid (Fig. 1).

The assembly of peptidoglycan on the outside of the plasma membrane is mediated by a group of periplasmic enzymes, which are transglycosylases, transpeptidases, and carboxypeptidases. These enzymes are designated as penicillin-binding proteins

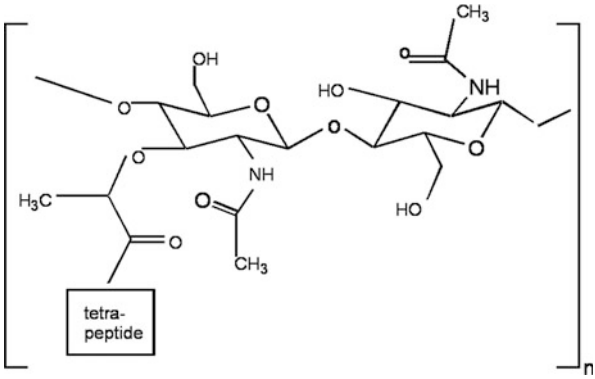

Fig. 1 Structure of peptidoglycan

(PBPs) being specific targets of the β-lactam antibiotics and are involved in the late stages of peptidoglycan synthesis which aids in the formation of a 3D mesh-like layer and a strong and rigid cell wall. Penicillin-binding proteins (PBPs) vary from species to species in number, size, amount, and affinity for β-lactam antibiotics. The high molecular mass PBPs (HMM-PBPs) mainly consist of two domain proteins that either belong to class A or class B, depending on the structure and the catalytic activity of their N-terminal domain (Heijenoort 2001). The C-terminal domain of both classes is responsible for transpeptidation activity, and β-lactam antibiotics covalently bind to its catalytic center. In class A HMM-PBPs, the N-terminal domain is responsible for their transglycosyltransferase activity, while, in the case of class B, the N terminal is involved in the interactions with other membrane proteins (Marrec-Fairley et al. 2000). It is well understood that class A HMM-PBPs are bifunctional enzymes capable of catalyzing both transglycosylation and transpeptidation.

The peptidoglycan layer is substantially thicker in the gram-positive bacteria (20–80 nm) as compared to gram-negative bacteria (7–8 nm) with the attachment of S-layer. Gram-positive bacteria are more sensitive to penicillin than gram-negative bacteria because the peptidoglycan is not protected by an outer membrane and is a more abundant molecule. In gram-positive bacteria, there are numerous different peptide arrangements among peptidoglycans. The best-studied murein is of *Staphylococcus aureus* wherein in place of diaminopimelic acid (DAP) as present in *E. coli* is diamino acid or L-lysine in position 3 of the tetrapeptide side chain, and the interpeptide bridge of amino acids connects a free amino group on lysine to a free carboxy group on D-Ala of a nearby tetrapeptide side chain. This arrangement allows a more frequent cross bonding between nearby tetrapeptide side chains. The free amino group of L-lysine is substituted with a glycine pentapeptide which then becomes an interpeptide bridge forming a link with a carboxy group from D-Ala in the adjacent tetrapeptide side chain as in the case of *S. aureus*. There are at least eight different types of peptidoglycan which exist in gram-positive bacteria and differ from species to species mainly with respect to the amino acids present in the third position of the tetrapeptide side chain and in the amino acid composition of the interpeptide bridge. The assembly of interpeptide bridge in gram-positive murein is inhibited by beta-lactam antibiotics (penicillin) in the same manner as the interpeptide bond in gram-negative murein.

Peptidoglycan forms 90 % of the dry weight of gram-positive bacteria but only 10 % for gram-negative strains. The cross-linked structure confers mechanical strength to cells and its desired shape in order to withstand internal osmotic pressure. It has sufficient plasticity to allow cell growth (elongation) and division (septation).

2.1 Biosynthesis of Peptidoglycan

The biosynthesis of peptidoglycan has been reported by various researchers in different microorganisms for both gram-positive and gram-negative bacteria (Matsuhashi 1994; Van Heijenoort 1998).

The peptidoglycan monomers are synthesized in the cytosol and are then attached to a membrane carrier bactoprenol. Bactoprenol transports peptidoglycan monomers across the cell membrane where they are inserted into the existing peptidoglycan. In the first step of the peptidoglycan synthesis, the glutamine, which is an amino acid, donates an amino group to sugar, fructose 6-phosphate. This converts fructose 6-phosphate into glucosamine-6-phosphate. In step two, an acetyl group is transferred from acetyl CoA to the amino group on the glucosamine-6-phosphate, creating *N*-acetylglucosamine-6-phosphate. In step three of the synthesis process, the *N*-acetylglucosamine-6-phosphate is isomerized, which will change *N*-acetylglucosamine-6-phosphate to *N*-acetylglucosamine-1-phosphate. In step four, the *N*-acetylglucosamine-1-phosphate, which is now a monophosphate, attacks the uridine triphosphate (UTP), which is a pyrimidine nucleotide and has the ability to act as an energy source. In this particular reaction, after the monophosphate has attacked the UTP, an inorganic pyrophosphate is released and is replaced by the monophosphate, creating UDP-*N*-acetylglucosamine. (When UDP is used as an energy source, it gives off an inorganic phosphate.) This initial stage is used to develop the precursor for the NAG in peptidoglycan. In step five, some of the UDP-*N*-acetylglucosamine (UDP-GlcNAc) is converted to UDP-MurNAc (UDP-*N*-acetylmuramic acid) by the addition of a lactyl group to the glucosamine. Also in this reaction, the C3 hydroxyl group will remove a phosphate from the alpha carbon of phosphoenolpyruvate. This creates what is called an enol derivative that will be reduced to a "lactyl moiety" by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in step six. In step seven, the UDP-MurNAc is converted to UDP-MurNAc pentapeptide by the addition of five amino acids, usually including the dipeptide D-alanyl-D-alanine. Each of these reactions requires involvement of the energy source Adenosine triphosphate (ATP). The above reactions are referred to as stage one. Stage two occurs in the cytoplasmic membrane. It is in the membrane where a lipid carrier called bactoprenol carries peptidoglycan precursors through the cell membrane. Bactoprenol will attack the UDP-MurNAc penta, creating a PP-MurNac penta, which is now a lipid. UDP-GlcNAc is then transported to MurNAc, creating lipid-PP-MurNAc penta-GlcNAc, a disaccharide and also a precursor to peptidoglycan. How this molecule is transported through the membrane is still not clear. However, once it is there, it is added to the growing glycan chain. The next reaction is known as transglycosylation. In this reaction, the hydroxyl group of the GlcNAc will attach to the MurNAc in the glycan, which will displace the lipid-PP from the glycan chain. The enzyme responsible for this reaction is transglycosylase (White 2007).

2.2 Inhibitors for Peptidoglycan Synthesis

Glycopeptides and moenomycins are best-studied groups of antibiotics which interfere with the transglycosylation reaction (Reynolds 1989) though their mode of action varies, but they have certain common features such as large molecular masses, no cell penetration, preferential in vivo action on gram-positive

microorganisms, and an accumulation of peptidoglycan precursors in intact cells and in cell-free systems. The non-penetration of such inhibitors further substantiates the localization of the polymerization reactions on the outside of the cytoplasmic membrane. Realizing this, Salton and Kim (1996) stated that there are certain antibacterial drugs such as penicillin which interferes with the production of peptidoglycan by binding itself to the bacterial enzyme such as transpeptidases or penicillin-binding protein or carboxypeptidases and thereby forming the loosely woven structure by inhibiting the cross-linking of tetrapeptide chain with another chain. Vancomycin binds or forms complex with D-Ala-D-Ala dipeptide of exported lipid II or with those of nascent peptidoglycan. It is either both mechanisms are functioning in vivo or one of them is prominent. In the first case, wherein antibiotic complexed with lipid II, the resulting segregation of the lipid substrate will lead to an arrest of the glycan chain elongation, while, in the second case, the binding with peptide subunits of growing chains can have a shielding effect by steric hindrance on both transglycosylation and transpeptidation reactions. While mutation in the gene which codes for transpeptidases or ligase leads to reduced interaction with an antibiotic, a strong and rigid 3D mesh-like layer is formed which could be due to the phenomenon known as antibiotic resistance (Spratt 1994).

In nature, human body synthesized the antibiotic lysozyme found in tears which breaks the β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNaC) in peptidoglycan primarily to destroy the bacterial cells. This phenomenon has been explored in research laboratories mainly to break the cell walls of gram-negative bacteria as lysozyme-treated bacterial cells are osmotically sensitive and easily disrupted by shearing. Homogenization followed by density gradient centrifugation separates the cytosol and inner membrane as well as outer membrane. Certain archaea have shown the presence of a similar layer of pseudopeptidoglycan or pseudomurein wherein the sugar residues are β-1,3-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNaC).

3 Teichoic Acids

Teichoic acid is a major class of cell surface glycopolymers and is found within the cell wall of gram-positive bacteria such as species in the genera *Staphylococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Corynebacterium*, and *Listeria* and appears to extend to the surface of the peptidoglycan layer. These are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars linked via phosphodiester bonds. There are two types of teichoic acid: the lipoteichoic acid (LTA) polymers which contain a reducing terminal phosphatidic acid, occasionally anchored to the plasma membrane and is extended from the cell surface into the peptidoglycan layer, and the wall TAs (WTAs) which are covalently attached to peptidoglycan and extended through and beyond the cell wall. All the teichoic acids bound to peptidoglycan appear to be linked by a conserved linkage unit consisting of ManNAc-GlcNAc-1-P linked to C6 of a MurNAc residue. The chemical structure of WTAs is composed of ManNAc-(β 1→4)-GlcNAc

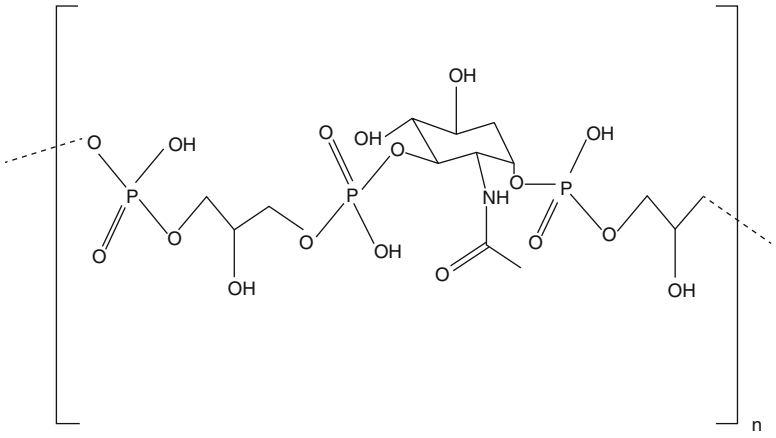

Fig. 2 Structure of teichoic acid

disaccharide with one to three glycerol phosphates attached to the C4 hydroxyl of the ManNAc residue (the "linkage unit") followed by a much longer chain of glycerol or ribitol phosphate repeats (the "main chain") (Fig. 2).

The exact function of teichoic acid in bacterial physiology is still not known though it is believed to be essential for the viability of gram-positive bacteria. There are certain evidences which elucidate the understanding of the important functions involved behind teichoic acids. The WTAs provide a channel of regularly oriented negative charges for threading positively charged substances through the complicated peptidoglycan network. The network of WTA-coordinated cations affects the overall structure of the polymers and this in turn influences the porosity and rigidity of the cell envelope. The WTA plays an important role in terms of cation homeostasis in gram-positive bacteria and provides a reservoir of ions close to the cell surface that might be required for enzyme activity (Marquis et al. 1976; Swoboda et al. 2010). In the past few years, researchers have tried to focus on the three-dimensional structure of WTAs and their bound cation group so as to elucidate the modes of cation binding to WTA polymer phosphate groups and to get an insight for designing the novel antimicrobials (Wickham et al. 2009). The other function is that wall teichoic acids (WTAs) are involved in the interaction of bacterial cells with the environment. WTAs in certain ways are involved in the regulation and assembly of muramic acid subunits on the outside of the plasma membrane. There are instances mainly for *Streptococci*, wherein teichoic acids have been involved in the adherence to the tissue surfaces. If these gram-positive bacteria lack or have defective WTAs, then there are a reduced initial adherence to artificial surfaces and also impaired ability to form biofilms. Contrary to other findings, Aly et al. (1980) mentioned in their study that impaired WTA does not exhibit reduced production of poly-*N*-acetylglucosamine (PNAG), the exopolysaccharide which is mainly involved in biofilm formation. Therefore, this suggests that WTAs played an independent role for biofilm formation. WTA is believed to be a virulent factor

and is required for host infection. Therefore, the enzymes involved in WTA biosynthesis were thought to be the targets for novel antimicrobials that could slow down colonization by gram-positive bacteria in the host cell. LTAs and WTAs have also shown to possess an important role in cell growth, division, and morphogenesis. In this context, Schirner and their coworkers indicated that in the absence of WTA expression, round and severely defective progeny is produced, while, during a defective gene expression of the biosynthesis of LTA, could hinder in septum formation and cell separation (Schirner et al. 2009). It was also suggested that the biosynthetic enzymes of WTA are associated with the machinery involved in elongation, while the biosynthetic enzymes involved for LTA might be associated with the machinery involved in septation and cell division.

3.1 Biosynthesis of Teichoic Acid

The pathway for WTA biosynthesis has been discussed here for *B. subtilis* 168 [synthesizes poly (glycerol phosphate) WTA], *B. subtilis* W23, and *S. aureus* [synthesizes poly (ribitol phosphate) WTA].

3.1.1 Biosynthesis of Poly (Glycerol Phosphate) WTA

Ward (1981) described that the pathway for the biosynthesis of poly (glycerol phosphate) WTAs was first characterized in *B. subtilis* 168. *tag* genes (also known as teichoic acid glycerol genes) were involved during biosynthesis. The pathway begins in the cytoplasm wherein the reversible enzyme TagO, which belongs to the family of phosphosugar transferases, could transfer GlcNAc phosphate to an undecaprenyl phosphate (also known as bactoprenyl phosphate) carrier which is anchored in the bacterial membrane. After catalyzing the reaction by TagO, the GlcNAc-PP-lipid is formed. Another enzyme, *N*-acetylmannosaminyl transferase (TagA), catalyzes the transfer of ManNAc from UDP-ManNAc to the C4 hydroxyl of the GlcNAc residue in order to form a β-linked disaccharide, which will now act as the substrate for the next enzyme in the pathway, TagB (Zhang et al. 2006). In order to complete the synthesis of the linkage unit, TagB, also known as glycerophosphate transferase enzyme, is involved which could transfer a single phosphoglycerol unit from CDP-glycerol to the C4 hydroxyl of ManNAc (Bhavsar et al. 2007). After the linkage unit formation, TagF (polymerizing cytidylyltransferase) could catalyze the attachment of 35 or more glycerol phosphates to the linkage unit in order to form an anionic polymer (Schertzer and Brown 2008). Once assembled, the lipid-linked WTA polymer is putatively modified by a glycosyltransferase (TagE) and then exported to the external surface of the bacterial membrane by a two-component ABC (ATP-binding cassette) transporter, TagGH (Lazarevic and Karamata 1995). The anomeric phosphate of the GlcNAc residue couples the polymer to peptidoglycan and esterified with D-alanine residues. The enzyme which could catalyze this reaction has till date not been identified though it belongs to the class of transferase enzyme.

3.1.2 Biosynthesis of Poly (Ribitol Phosphate) WTA in *B. subtilis* W23

Lazarevic and coworkers studied the pathway for the biosynthesis of poly (ribitol phosphate) WTA in *B. subtilis* W23 and reported that the genes involved were designated as *tar* genes (for teichoic acid ribitol) (Lazarevic et al. 2002). The initial three steps which were mediated by TarO, TarA, and TarB were identical to those in *B. subtilis* 168. Thereafter, pathways diverged wherein the TarF acts as primase and adds one additional glycerol phosphate unit to the 168-type linkage unit. Once the linkage unit is complete, the poly (ribitol phosphate) main chain is assembled. Lazarevic et al. proposed that the assembly of this poly (ribitol phosphate) chain requires two enzymes: TarK, which transfers a single ribitol phosphate residue to the linkage unit, and TarL, which carries out the polymerization of the ribitol phosphate chain. TarK and TarL in *B. subtilis* W23 were thus suggested to function as a primase/polymerase pair, analogous to the primase/polymerase pair (TagB/TagF) that assembles the poly (glycerol phosphate) chain in strain 168. Once the poly (ribitol phosphate) WTA polymer is assembled, the remaining steps are thought to be similar to those in strain 168. That is, the WTA polymer is glycosylated, transported through the bacterial membrane by a two-component transporter, TarGH, attached to peptidoglycan by an unidentified transferase, and esterified with D-alanine residues.

3.2 Inhibitors for Wall Teichoic Acid Biosynthesis

In the past few years, speculations are on that WTA biosynthetic pathway could be the site for the antibiotic, though still only one specific inhibitor has been reported (Swoboda et al. 2010). Swoboda and coworkers also stated that there are two distinct types of antimicrobial targets in the pathway: (1) antivirulent targets (TarO and TarA) and (2) antibiotic targets due to a mixed gene dispensability pattern. The inhibitors of the former slow down colonization and spread of infection, while inhibitors of the latter have shown to prevent bacterial growth. In this context, the inhibitors were evaluated for both target points.

Weidenmaier and coworkers were the first to suggest that the pathway for WTA biosynthesis is the site for antivirulence and had studied it for the case of *S. aureus* (Weidenmaier et al. 2004). Several researchers reported that there is a potent natural product inhibitor, the uridine-containing antibiotic tunicamycin for WTA biosynthesis (Hancock et al. 1976; Wyke and Ward 1977). Tunicamycin inhibits MraY, an enzyme, phosphosugar transferase activity (involved in the peptidoglycan biosynthetic pathway) which couples sugar phosphates to membrane-embedded lipid phosphates. Besides this, tunicamycin also inhibits TarO (Price and Tsvetanova 2007). It has been observed that tunicamycin is more selective for TarO over MraY, and due to this selectivity, it could shut off the in vitro WTA expression without affecting bacterial growth rates. Till date, researchers could not develop any other specific inhibitor or its homologue which could be used in place of tunicamycin for in vivo studies as this inhibitor is toxic to eukaryotes, so this strategy of inhibiting TarO gene involved during WTA biosynthesis could not be

worked out for treating *S. aureus* infections in humans. Therefore, there is a need to identify the nontoxic, selective inhibitors of TarO gene which are involved during the WTA biosynthetic pathway in order to cure different diseases.

The next target in the WTA biosynthetic pathway is the antibiotic target which was reported by Swoboda and coworkers (2010). Peschel and coworkers reported that *S. aureus* strains lacking WTAs are incapable of colonizing a host; however, these resistant mutants are not expected to survive in vivo (Peschel et al. 1999). But, Swoboda et al. pointed out that there are numerous other pathways that contain conditionally the essential enzymes linked to virulence factor expression. Many of these enzymes could be good antibiotic targets provided that the major mechanism for resistance involves deletion of the pathway and results in the production of avirulent organisms (Swoboda et al. 2010).

4 Bacterial Glycoproteins

Glycans are an important constituent of bacterial cell wall and of surface antigens such as lipopolysaccharides (LPSs) and capsular antigens which are attached by lipid anchors (lipid A or diacylglycerophosphates) in gram-negative bacteria (Gotschlich et al. 1981; Schmidt et al. 2003). These carbohydrate conjugates function as virulent factors which have been well documented in literature, while the structure-function relationships of bacterial cell surface lipoglycans have been elucidated. It aids in maintaining structural integrity and correct protein localization and in the induction of host responses (Beutler and Rietschel 2003).

In the past few decades, researchers thought that prokaryotes are incapable of glycosylating the proteins. But now, researchers have firmly established that there is a glycosylation of proteins in eubacteria which is due to the glycoprotein synthetic pathway being operated inside the prokaryotic cell (Power and Jennings 2003; Upreti et al. 2003). The protein glycosylation has initially been reported in Archaea and later in *Clostridia* which exhibited the glycosylated surface (S) layer proteins (Schaeffer and Messner 2001). Earlier, the presence of glycoproteins in bacteria was detected either through the formation of Schiff bases or by the specific binding of lectins. The type of glycosylation either N or O type could be distinguished by using endoglycosidases F (Endo F1, F2, F3, and D), PNGase F, or O-glycosidase in combination with the inhibitors such as bacitracin or tunicamycin which inhibits glycosylation.

4.1 Importance of Protein Glycosylation in Gram-Negative Bacteria

4.1.1 Flagellar Glycosylation and Motility

The *N*- or *O*-glycosylation of the peptide in bacteria could induce the host response by causing infection. In this context, in order to study the effect of protein glycosylation in bacteria on host's mechanism, *Campylobacter* spp. is considered

to be a prominent example as it is believed to be an agent which generally causes diarrhea. The flagellin, an immunodominant protein, is recognized as an essential constituent in flagella which could act as a virulence factor. The flagellins of *C. jejuni* and *C. coli* were found to be extensively glycosylated as reported by Guerry and coworkers (1996). In the case of *C. jejuni*, the glycoprotein structure has been revealed by Szymanski et al. wherein it was indicated that the carbohydrate structure has pseudaminic acid or its derivatives Pse5Am7Ac and Pse5Pr7Pr, which are linked to approximately 19 serine or threonine residues. These immunogenic substituents are clustered in the hydrophobic central core region, and the surface is exposed with flagella filaments (Szymanski et al. 2003). Reports suggest that adjacent to the flagellin structural genes in the genome of *C. jejuni* (NCTC 11168) lies the locus containing approximately 50 genes which represents the flagellin glycosylation machinery (Parkhill et al. 2000).

The other protein glycosylation locus (pgl) of 17 kb mediates the *N*-glycosylation of multiple proteins in *C. jejuni* and represents the first such pathway in gram-negative pathogens (Young et al. 2002). However, mutation in pgl genes affects glycosylation and also abolishes the recognition by polyclonal rabbit antiserum and by antibodies from humans which were infected with *C. jejuni*. Furthermore, insertional inactivation of pgl genes resulted in decreased adherence, invasion in vitro, and loss of intestinal colonization in mice (Szymanski et al. 2002). In addition to the nonflagellar glycoproteins PEB3, an immunoreactive surface protein (Cj0289c; Cj1670c) along with 22 periplasmic glycoproteins was identified. All the glycoproteins have a common oligosaccharide structure a-D-Galp-NAc-(1-4)-a-D-GalpNAc-(1-4)-a-D-GalpNAc [b-D-Glcp-(1-3)]-(1-4)-a-D-GalpNAc-(1-3)-b-D-Bac-(1,N)-Asn Xaa, where Bac is bacillosamine (2,4-diacetamido-2,4,6-trideoxyglucopyranose). Bacillosamine has also been found to be a constituent of LPS and capsular polysaccharide in other prokaryotes (Schaeffer et al. 2001; Schmidt et al. 2003). Szymanski and coworkers reported that PglB is a key enzyme in the pgl locus and probably acts as an oligosaccharide transferase mediating the N-linkage to the peptide backbone; however, any mutation in the pglB gene could totally abolish the glycosylation (Szymanski et al. 2003).

Motility is an important factor in *Helicobacter*-associated diseases. Flagella of gastric *Helicobacter* spp. (*H. pylori*, *H. mustelae*, and *H. felis*) exhibit two distinct flagellins, FlaA and FlaB. Mutants lacking FlaA have impaired motility and shortened flagella, while deletion of FlaB gene has shown only moderate effect (Schmidt et al. 2003).

4.1.2 Pili and Adhesions in Gram-Negative Bacteria

There are several gram-negative microorganisms wherein protein glycosylation in pili influences pathogenesis. Certain examples have been discussed here in this chapter.

In the case of *Haemophilus influenzae*, the infection is being caused through its attachment to the human epithelial cells which occurs by two related adhesions HMW1 and HMW2 being encoded by hmw1 and hmw2, and both genes are associated with hmwB and hmwC (Schmidt et al. 2003). Grass et al. described

that HMW1 is glycosylated and that HMWIC is needed for glycosylation. It was observed that the glycan moiety of HMW1 contains galactose (Gal), glucose (Glc), and mannose (Man) residues and requires the activity of phosphoglucomutase, which is involved in the lipooligosaccharide (LOS) biosynthesis pathway. Shedding of the non-glycosylated adhesins decreases the adherence to epithelial cells. Therefore, glycosylation directly modulates cellular interactions and might also influence pathogenesis (Grass et al. 2003).

In *Neisseria*, type IV pili aid in bacterial attachment to the host cell through the glycosylation of adhesins. Stimson et al. (1995) revealed that in *N. meningitidis* strain C311, there is an O-linked glycan substitution at serine residue 63 (Ser63) wherein pglA or pgtA (pilin glycosylation) gene could transfer the first a-(1, 3)-linked galactose to the diacetamido trideoxyhexose Ser63-substituent. On the other hand, certain researchers observed that strains which are deficient in pilin glycosylation could express more type IV pili along with an increased adherence to the epithelial cells but less resistance against solubilization were present (Marceau et al. 1998). Parge and coworkers deduced the structure of glycoprotein through electron density maps as a-Gal-(1, 3)-GlcNAc disaccharide which is being linked to Ser63 in *N. gonorrhoeae* strain MS11 pili (Parge et al. 1995). The homopolymeric guanosine tract identified in certain strains is basically involved in the phase variation of pglA expression which is associated with the conversion from uncomplicated gonorrhea to disseminated gonococcal infections (Banerjee et al. 2002).

4.1.3 Glycosylation of Surface and Membrane Proteins

Borrelia burgdorferi is the causative agent of Lyme disease. In this microbe, the major surface (lipo) proteins, i.e., OspA and OspB (31 and 34 kDa, respectively), are differentially expressed during the infectious cycle. However, at the time of infection, the presence of anti-Osp antibody prevents the disease by largely reducing or killing the spirochete population in the tick before transmission to the host (Schwan and Piesman 2002). In the present scenario, the human vaccine is based on OspA.

4.1.4 Pili and Adhesion in Gram-Positive Bacteria

Streptococcus parasanguis, being a gram-positive bacterium, colonizes on the tooth surface and plays a pivotal role in the development of dental plaque. Adhesion is mediated by the major fimbrial subunit Fap1, wherein 80 % of the Fap1 protein consists of dipeptide serine repeats. The glycopeptide showed the presence of Rha (rhamnose), Glc, Gal, GlcNAc, and GalNAc. The adherence properties of Fap1 are dependent on its glycan modification because monoclonal antibodies that blocked adhesion did not recognize recombinant Fap1, but were inhibited by a Fap1-derived glycopeptide and showed reduced binding to periodate-oxidized Fap1 (Stephenson et al. 2002).

4.1.5 Glycosylation of Cell Wall and Secreted Proteins

Streptococcus mutans is largely responsible for human dental caries and occasionally causes infective endocarditis in predisposed patients. An immunodominant cell

wall-associated glycoprotein of 60 kDa (IDG-60) carrying NeuNAc (*N*-acetylneuraminic acid), Man, and Gal residues has been identified by screening a genomic library of *S. mutans* strain GS-5. This protein is essential for maintaining cell wall integrity and cell shape; however, the role of the glycan modification has not been elucidated (Chia et al. 2001).

4.1.6 Molecular Mimicry of Eukaryotic Glycoproteins by Bacteria

With time, bacteria have developed glycoconjugates which are closely related to those found in mammals. *Chlamydia trachomatis* synthesizes high-mannose-type *N*-glycan on glycoprotein which is similar to the type assembled on eukaryotic glycoproteins which also produce heparin-like molecule causing infection in the eukaryotic host. *Nisseria meningitidis* produces adhesive fimbriae that contain O-glycan in a pili subunit. The assemblies of eukaryotic-like glycans by pathogenic bacteria may be a form of molecular mimicry for bacteria in order to avoid or to compromise the immune system of the host (Varki et al. 1999).

5 Membrane-Derived Oligosaccharides

Membrane-derived oligosaccharides (MDOs) were named due to the membrane origin of the phosphoglycerol substituents. These are present in the periplasmic space and are widely distributed in gram-negative bacteria. These are also known as the osmoregulated periplasmic glucans (OPGs) since the synthesis and accumulation of MDOs are inversely proportional to the osmotic strength of the environment (Bohin 2000). MDOs are a heterogeneous family of closely related, highly branched oligosaccharides containing glucose as the sole sugar. They are substituted with sn-1-phosphoglycerol, phosphoethanolamine, and 0-succinyl ester residues, giving these molecules a net negative charge. MDOs contain 6–12 glucose residues joined by K-1,2 and K-1,6 linkages, with the principal species containing eight to nine glucose units (Kennedy 1996). Several reports suggest the important functions of MDOs in *E. coli*, such as its role in cell to cell signaling, chemotaxis, lysis induction by bacteriophages, the regulation of capsular polysaccharide synthesis, and the osmoregulation of the outer membrane protein expression (Geiger et al. 1992). Bohin and coworkers stated that when *E. coli* cells were grown in low-osmolarity medium, the MDOs in the periplasmic space represent 3.5–5 % of the cell dry weight, while the cells when grown in the medium with higher osmolarity exhibit a drastic decrease in MDO content (Bohin 2000; Bohin and Lacroix 2006).

5.1 Biosynthesis of MDOs

Limited reports are available on the biosynthesis of MDOs; however, the pioneering study was reported by Dedonder and Hassid (1964). In the past decade,

researchers investigated the synthesis of cyclic glucans in *Rhizobiaceae* and established that membrane-bound glucosyltransferase system catalyzes an essential step in the biosynthesis of MDOs wherein glucose residues are transferred from UDP-glucose to octyl β-D-glucoside (acceptor) with the formation of β 1→2-linked polyglucose chains linked to the acceptor molecule (Weissborn and Kennedy 1984). It was also reported that any mutation in the mdoA locus leads to the loss of membrane-bound glucosyltransferase activity and thereby prevents the formation of MDO chains in vivo. Besides the membrane fraction, a soluble protein identified as acyl carrier protein (ACP) from the cytosolic fraction is also required during the process of biosynthesis. Jackson and Kennedy (1983) proposed a two-step model wherein phosphoglycerol transferase I, an enzyme of the inner membrane, catalyzes the transfer in vitro phosphoglycerol residues from phosphatidylglycerol to nascent MDOs (membrane-linked newly synthesized glucans) or to the synthetic β-glucoside acceptors such as arbutin. Then, the periplasmic phosphoglycerol transferase II would transfer those residues from one molecule of OPG (potentially still an acceptor in the first step) to another already liberated in the periplasmic space. The sn-1,2-diglyceride that is the product of this reaction is normally phosphorylated by the enzyme diglyceride kinase in a salvage reaction, leading to the formation of phosphatidic acid, which then can be used for the synthesis of cellular phospholipids (Jackson et al. 1984).

5.2 Inhibitors of MDO Biosynthetic Pathway

The membrane-bound transglucosylase system is inhibited in a dose-dependent manner by antibiotics such as bacitracin and amphomycin. These antibiotics generally function in a cell-free transglucosylase enzyme system by forming specific complex with polyisoprenyl phosphate derivatives (Banerjee 1989).

6 Lipopolysaccharides

A lipopolysaccharide (LPS) or lipoglycan molecule is made up of a lipid and a polysaccharide which are joined together by a covalent bond. LPSs are present in the outer membrane of the gram-negative bacteria. The low molecular weight form of bacterial polysaccharides is known as lipooligosaccharides (LOSs). The toxicity of LPS was discovered by Richard Friedrich Johannes Pfeiffer and termed as endotoxin. It was reported that endotoxins are toxins released by the bacteria into the environment and are kept within the bacterial cells and are released only after bacterial cell wall destruction (Parija 2009). Researchers used the term endotoxin as a synonym with LPS. The important function of LPS is to provide structural integrity to bacteria and its membrane protection from certain kinds of chemical attack. LPS also aids in membrane stabilization by increasing the negative charge on the cell membrane. This molecule is also believed to be a molecule of life for the

gram-negative bacteria since any sort of mutation or removal could lead to its death. LPS could also illicit a strong response from normal animal immune system. Besides its pathogenic nature, the nonpathogenic aspects have also been studied such as surface adhesion, bacteriophage sensitivity, and interactions with predators such as amebae.

Lipopolysaccharide (LPS) comprises of three parts: (i) "O" antigen, (ii) core polysaccharide, and (iii) lipid A.

6.1 "O" Antigen

It is also named as "O" antigen, "O" polysaccharide, or "O" side chain of the bacteria; it is basically a repetitive unit of glycans and forms a polymer. It is exposed on the very outer surface of the LPS molecule and is attached to the core oligosaccharide in a bacterial cell. The composition of "O" chain varies from strain to strain. Rittig et al. (2004) mention two types of LPS, smooth and rough on the basis of the presence or the absence (or reduced form) of "O" chain respectively. It was also observed that bacteria with rough LPS are more hydrophobic and have a more penetrable cell membrane for hydrophobic antibiotics (Tsujimoto et al. 2003).

6.2 Core Polysaccharide

The core generally contains the oligosaccharide component that attaches itself to lipid A. The sugars commonly present are heptose and 3-deoxy-D-mannooctulosonic acid (also known as KDO, ketodeoxyoctulosonate) (Hershberger and Binkley 1968). In most of the bacteria, a noncarbohydrate component is also present in the LPS core such as phosphate, amino acids, and ethanolamine substituents. In the case of *Neisseria meningitidis*, the inner core is composed of 3-deoxy-D-manno-2-octulosonic acid (KDO) and heptose (Hep) moieties.

6.3 Lipid A

Generally, the lipid A moiety is a much conserved component of the LPS. It is a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is mainly responsible for much of the toxicity of gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock) (Tzeng et al. 2002).

6.4 Lipooligosaccharides (LOSs)

These are the glycolipids which are present in the outer membrane of certain types of gram-negative bacteria such as *Neisseria* spp. and *Haemophilus* spp. It is basically a low molecular weight lipopolysaccharide. LOS helps in maintaining the structural integrity and functionality of the outer membrane in gram-negative microorganisms. Lipooligosaccharides act as immunostimulators and immunomodulators; therefore, its important role in the pathogenesis of certain bacterial infections has been reported (Moran et al. 1996). In certain bacterial strains, LOS molecules display molecular mimicry and antigenic diversity, aiding in the evasion of host immune defenses and thus contributing to the virulence of these bacterial strains. In contrast to lipopolysaccharides, lipooligosaccharides lack "O" antigens and possess only a lipid A-based outer membrane-anchoring moiety and an oligosaccharide core (Kilár et al. 2013).

6.5 LPS Modifications

With time, the host system could manage the toxic effects of gram-negative bacteria which are generally present in the small intestine. In the past few years, it has been reported that a specific enzyme in the host's intestine, i.e., alkaline phosphatase, could detoxify the toxic effect caused by lipopolysaccharide through the removal of two phosphate groups being present on the LPS carbohydrates (Bates et al. 2007). The other route for detoxification could be the addition of more specific sugars using glycosyltransferases in order to modify the structure of LPS.

6.6 Biosynthesis of Lipopolysaccharides

It has been observed that in *Salmonella* all *wbaP* (*rfbP*) genes encode for an enzyme (glycosyltransferase) that transfers Gal-1-P to undecaprenol-P as an initiating step of "O" units, and all have sequences that are readily aligned. It has also been reported that in certain *Salmonella* strains like from group D and group B strains, gene *wbaV* (*rfbV*) encodes for tyvelose transferase and an abequose transferase respectively, and each could carry out functions in the presence of appropriate precursors.

The genes for saccharide processing (including export, polymerization, and assembly of complex polysaccharides such as LPS) commonly occur in families of homologous genes that perform same general functions. Two genes with the same name may have specificity for different oligosaccharides and are distinguished by including the species or some other relevant information as subscript.

Most "O"-antigen gene clusters have genes currently known as rfc, rfbX and cld, or rol. Each could be recognized by the topological features of the encoded protein

although there is a certain sequence similarity for the proteins in the first two families. The wzy genes are generally found within the "O"-antigen gene cluster (Reeves et al. 1996).

6.7 Functions of Lipopolysaccharides

6.7.1 Biological Effects of Lipopolysaccharides on Hosts

Lipopolysaccharides in gram-negative bacteria usually act as a candidate target for new antimicrobial agents. LPS generally acts as endotoxin as it binds to the cell receptor (CD14/TLR4/MD2) complex in monocytes, dendritic cells, macrophages, and B cells, which thereby promotes the secretion of proinflammatory cytokines, nitric oxide, and eicosanoids (Abbas 2006). The TLR4 as the LPS receptor was demonstrated by Bruce Beutler, a noble laureate for this work in the year 2011 in Physiology or Medicine (www.nobleprize.org/nobel_prizes/medicine/laureates/2011/press.html). LPS also acts as an exogenous pyrogen or as the external fever-inducing substance. The functions of LPS are under experimental research for several years as it activates many transcription factors. LPS produces different types of mediators which are involved in septic shock. In this context, Warren et al. (2010) observed that humans are more sensitive to LPS as compared to other animals and reported that a minimal dose of 1 μg/kg induces shock in humans, wherein mice could tolerate a dose which is a thousand times higher compared to the humans' minimal dosage. This difference could be due to the level of circulating natural antibodies (Reid et al. 1997).

Endotoxins could aggravate the pathogenic effect by *Neisseria meningitidis*, a gram-negative microorganism, and could cause meningococcal disease, including meningococcemia, Waterhouse-Friderichsen syndrome, and meningitis.

Certain portions of LOS in some bacteria have shown similarity in terms of their chemical structure with the human host cell surface molecules. It is termed as **molecular mimicry**. Moran and coworkers observed and reported that in the case of certain strains of *Neisseria meningitidis*, the terminal tetrasaccharide portion of the oligosaccharide (lacto-*N*-neotetraose) is the same tetrasaccharide as that found in paragloboside, a precursor for ABH glycolipid antigens found on human erythrocytes. Furthermore, the terminal trisaccharide portion (lactotriaose) of the oligosaccharide from the pathogenic *Neisseria* spp. is also present in the lactoneoseries of glycosphingolipids from human cells. Besides this, the presence of these human cell surface mimics may also act as a camouflage from the immune system and could abolish the immune tolerance when infecting hosts with certain human leukocyte antigen (HLA) genotypes, such as HLA-B35 (Moran et al. 1996).

6.7.2 Effect of LPS Variation on Immune Response

The outer carbohydrate portion or "O" antigen is believed to be the most variable portion of the LPS molecule, and this thereby imparts the antigenic specificity. In contrast, lipid A portion is the most conserved part in LPS, but this portion could also vary in number and nature of acyl chains present within or different genera. These variations in lipid A could impart antagonistic properties to LPS like in the case of

Rhodobacter sphaeroides, wherein diphosphoryl lipid A (RsDPLA) is a potent antagonist of LPS in human cells but is an agonist in hamster and equine cells. In the normal human blood serum, anti-LOS antibodies are present which are bactericidal, while the patients who are suffering from infections arising out of serotypically distinct strains possess anti-LOS antibodies that differ in their specificity primarily within the structure of the oligosaccharide portion of the LOS molecule (Yamasaki et al. 1994). During infection, the variation in the antigenicity of LOS was observed which could be due to the ability of bacteria to synthesize more than one type of LOS molecule through sialylation, which might lead to an increase in resistance to complement-mediated killing (Yamasaki et al. 1994) or can down-regulate the complement activation (Moran et al. 1996) or even it can evade the effects of bactericidal antibodies.

6.7.3 Health Effects of Lipopolysaccharide on Host

The presence of endotoxins in the blood is called endotoxemia. It could lead to septic shock, if the immune response is severely pronounced (Opal 2010). Endotoxemia in the intestine could lead to the development of alcoholic hepatitis (Ceccanti et al. 2006). It has also been reported that gonococcal LOS can cause damage to the human fallopian tubes.

The molecular mimicry of some LOS molecules is thought to cause autoimmune-based host response. The bacteria's mimicry to the host structures via LOS is found with *Helicobacter pylori* and *Campylobacter jejuni*, the organisms which cause gastrointestinal disease in humans, and *Haemophilus ducreyi* which causes chancroid. Certain *C. jejuni* LPS serotypes (attributed to certain tetra- and pentasaccharide moieties of the core oligosaccharide) have also been implicated with Guillain-Barré syndrome and a variant of Guillain-Barré called Miller Fisher syndrome (Moran et al. 1996).

An increase in the endotoxin load due to an increased population of endotoxin-producing bacteria in the intestinal tract is associated with obesity-related patients. This clearly shows that the presence of a high level of endotoxin from *Escherichia coli* could induce obesity as has been observed by Cani and coworkers through experimental studies wherein they injected purified endotoxin from *Escherichia coli* into germ-free mouse models (Cani et al. 2007). It has also been reported that endotoxin is associated with obesity because it induces an inflammation-mediated pathway. Recently, it was observed that *Enterobacter cloacae* B29 could also contribute toward the obesity and insulin resistance in human patients (http://www.nature.com/ismej/journal/vaop/ncurrent/full/ismej2012153a.html).

Endotoxins could be detected through the standard assay known as limulus amebocyte lysate (LAL) assay utilizing the blood from the horseshoe crab (Iwanaga 2007).

7 Capsular Polysaccharide

The capsular polysaccharide constitutes the outermost layer of the bacterial cell and mediates its direct interaction with the environment. Due to these interactions, polysaccharide capsules have been implicated as an important virulence factor for

many bacterial pathogens. The capsular polysaccharide is mainly found in gram-negative bacteria such as in *E. coli*, *Neisseria meningitidis*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Salmonella*. In certain gram-positive bacteria, these capsular polysaccharides were also observed such as in the case of *Bacillus megaterium* (could synthesize a capsule composed of polypeptide and polysaccharides), *Streptococcus pyogenes* (synthesizes hyaluronic acid capsule), *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Staphylococcus epidermidis*. Besides this, the capsular polysaccharide was also observed in yeast particularly in *Cryptococcus neoformans* which has a capsular polysaccharide similar to that of bacteria. Generally, it is very difficult to distinguish between the capsular polysaccharide (CPS) and other surface polysaccharides, such as the "O"-antigenic moieties of lipopolysaccharide (LPS), as CPS is found to be associated with LPS (Whitfield 1988). The capsule could be identified either through India ink staining or through serological methods wherein the capsular material acts as antigen and is mixed with specific anticapsular sera. When it was observed under a microscope, the capsule appears swollen due to an increased refractivity. This phenomenon is known as Quellung reaction.

Capsular polysaccharides are the hydrated molecules that have more than 95 % water. These molecules are linked to the bacterial cell surface via covalent attachment to either phospholipids or lipid-A molecules, while, in certain cases, it was observed that CPS may be associated directly with the cell without any membrane anchor (Whitfield 1988; Whitfield and Valvano 1993). The capsular polysaccharide could either be homo- or heteropolymers composed of repeating monosaccharides joined by glycosidic linkages (Roberts 1996). The two monosaccharide units may be joined in a number of configurations which may lead to a large structural diversity in CPS among species, while chemically identical capsular polysaccharides may also be synthesized by different bacterial species. It was identified that certain CPS or K antigen is associated with certain infections. The *Escherichia coli* K1 antigen, a homopolymer of α2,8-linked *N*-acetylneuraminic acid, is the major cause of neonatal meningitis (Robbins et al. 1974). The capsular polysaccharide of *Neisseria meningitidis* group B is identical to the K1 polymer of *E. coli* (Grados and Ewing 1970).

The bacterial capsular polysaccharide could perform a number of functions such as prevention of desiccation, adherence, resistance to nonspecific host immunity, and resistance to specific host immunity and thereby mediating the diffusion of molecules through the cell surface (Whitfield 1988; Roberts 1996). The encapsulated bacteria could sustain or survive outside the host and thereby promotes the transmission of pathogenic bacteria from one host to another; it could be due to the formation of hydrated gel around their cell surface which protects the cell from the effects of desiccation. Capsular polysaccharide promotes biofilm formation as this molecule has the tendency to adhere the bacterial cell either to the surface or with other bacterial cells which facilitates the colonization (Costerton et al. 1987).

During the invasive bacterial infections, interactions between the capsular polysaccharide and the host's immune system can decide the outcome of the infection

(Roberts et al. 1989; Roberts 1996). In the absence of specific antibody, the presence of capsule confers resistance to the nonspecific host defense mechanisms. These responses include the activation of the complement cascade via the alternative pathway and of the C3b-mediated opsonophagocytosis by polymorphonuclear leukocytes. Both these responses provide protection in the preimmune host when specific antibodies are absent. The alternative pathway is initiated by the binding of C3b to the bacterial cell surface. The bound C3b is activated by factor B and thereby forms C3convertase C3bBb. This now allows the binding of more C3 and the formation of membrane attack complex on the outer membrane of the bacteria which leads to lysis and death (Finne 1982; Roberts 1996). This phenomenon could be inactivated by capsule which could provide a cell permeability barrier to complement components, thereby masking the C3b deposited on cell surface structures from C3b receptors on the phagocyte cell surface that could otherwise be potent activators of the alternative pathway (Howard and Glynn 1971). The net negative charge is being conferred on to the cell surface by the capsular polysaccharide in order to confer resistance toward phagocytosis. The net charge is directly proportional to the conferred resistance toward phagocytosis.

In most of the cases, capsular polysaccharides could elicit an immune response while certain are poorly immunogenic. The poor immunogenicity has been observed in *E. coli* KI capsule, *E. coli* K5 capsule, and *Neisseria meningitidis* serogroup B primarily due to the presence of NeuNAc in the capsules which is similar to desulfoheparin (Bhattacharjee et al. 1975). These capsules are structurally similar to the polysaccharides encountered on host tissue; therefore, these capsules are poorly immunogenic and thereby elicit a poor antibody response in the host (Wyle et al. 1972). *Burkholderia pseudomallei* is believed to be the causative agent of melioidosis which is due to direct inoculation into wounds and skin abrasions or due to the inhalation of a contaminated material. This disease is present as an acute pneumonia or as an acute septicemia, a more severe form of disease. The chronic and the subclinical forms generally remain undetected until activated by a traumatic event or decrease in immunocompetence (Ip et al. 1995). *B. pseudomallei* is resistant to a large number of antibiotics; even with the aggressive antibiotic therapy, the mortality rate remains high and the incidence of relapse is common (Currie et al. 2000). Reckseidler-Zenteno reported that certain cell-associated antigens were identified in *B. pseudomallei*. The cell-associated antigens include capsular polysaccharides and lipopolysaccharides (Reckseidler-Zenteno 2012). The capsular polysaccharide produced by *B. pseudomallei* is determined to be an unbranched polymer of repeating tetrasaccharide units with a structure -3)-2-O-acetyl-β-D-Galp-(1-4)-α-D-Galp-1-3)- β-D-Galp-(1-5)- β-D-KDOp-(2- (Masoud et al. 1997). The exact role of CPS in terms of virulence was not known but the sera of patients suffering with melioidosis have shown the presence of antibodies against CPS. The two other CPS structures were also identified: a branched 1,4-linked glucan polymer (CP-1a) and a triple-branched heptasaccharide repeating unit composed of rhamnose, mannose, galactose, glucose, and glucuronic acid (CP-2) (Perry et al. 1995). The genes involved in the synthesis of these capsules and the role of these capsules in virulence had not been identified.

7.1 Biosynthesis of Capsular Polysaccharide

The capsular polysaccharide is produced by many gram-negative bacteria by one of the two assembly systems, i.e., either by Wzy-dependent pathway or ATP-binding cassette (ABC)-transporter-dependent pathway (Cuthbertson et al. 2009; Willis and Whitfield 2013).

Among the gram-negative bacteria, the capsular K antigens of *E. coli* are widely studied, and the capsule system is differentiated into four groups based on assembly system, key genes organization, and regulatory features (Whitfield 2006). The Wzy-dependent pathway is used for groups 1 and 4 capsules wherein the isolates are associated with gastrointestinal infections. In this pathway, the initial synthesis took place in the cytoplasm wherein the repeating units of polyprenol-linked CPS are synthesized which are then flipped by a Wzx protein across the inner membrane and further polymerized into the full length CPS by a Wzy protein. The CPS chains are assembled into a capsular structure onto the cell surface, but how it is retained in the cell surface is not entirely clear though it is a multifactorial process. The similar process exists in genera such as *Klebsiella* and *Erwinia*.

The capsular system of groups 2 and 3 in *E. coli* requires ABC-transporter-dependent assembly. This system is found in mucosal pathogens which include *Campylobacter jejuni*, *N. meningitidis*, *H. influenzae*, *Mannheimia haemolytica*, etc. These bacteria caused variety of diseases in humans like septicemia, meningitis, urinary tract infections, gastrointestinal infections, and otitis media (Agrawal and Murphy 2011). The CPS assembled by the ABC-transporter-dependent pathway is in the presence of phospholipids at the reducing end of the polysaccharide chain. Willis and Whitfield (2013) reported that the terminal lipid is attached to the repeating units of polysaccharide chain in *E. coli* K1 and K5 to the cell surface. The phospholipid is lyso-phosphatidylglycerol (PG) which is attached to the CPS chain via a novel β-linked poly-3-deoxy-D-manno-oct-2-ulosonic acid (kdo) linker. The amount of CPS associated with the cell is very difficult to measure since the linkage between the lipid and CPS is unstable; however, other mechanisms are also there which allow the CPS to link to the cell membrane such as the ionic interactions between CPS and the core region of LPS as observed in *E. coli* K1 (Jimenez et al. 2012).

In the ABC-transporter-dependent pathway, CPS is synthesized in the cytoplasm wherein the repeating units of polysaccharide structure are assembled through the action of glycosyltransferase (GT) wherein GTs catalyze the transfer of specific sugars from an activated donor to the non reducing end of the growing CPS glycan (DeAngelis 1999) and are exported across the inner membrane via an ABC transporter comprising of two identical nucleotide-binding domain (NBD) polypeptides (KpsT in *E. coli*) and two integral membrane polypeptides (KpsM). In order to complete the transportation from the periplasm toward the cell membrane, it requires two other characteristic components: a member of the polysaccharide co-polymerase (PCP-3) family (KpsE) and an outer membrane polysaccharide (OPX) protein (KpsD). These proteins are thought to form protein complex, enabling synthesis and translocation from the cytoplasm to the cell surface in a coordinated process.

KpsE, being a PCP family protein, is anchored to the inner membrane with N- and C-terminal transmembrane helices. KpsE interacts with KpsD, the OPX family protein, and this interaction is specific for the cognate pairs of KpsED homologues. In *E. coli* group 1 CPS form, the OPX protein forms an octameric outer membrane channel which could facilitate the CPS efflux. The OPX protein of CPS group 1 is mainly located in the periplasm wherein it interacts with the periplasmic domain of its cognate PCP-2a protein. The KpsD and its homologues possess a motif shared by all the OPX proteins, but they do not form stable multimers in the outer membrane so their 3D structures may differ from group 1 OPX protein. The efflux pump is organized in a tripartite and aids in the CPS export system. Here, the efflux pump is connected to an outer membrane channel such as ToIC via an adaptor protein in order to create a contiguous channel from the cytosol to the exterior of the cell (Willis and Whitfield 2013).

A lot more studies need to be carried out to understand the complete mechanism for CPS biosynthesis, since there is a need to understand the mechanism as to how the substrate for the polymerizing CPS synthases is synthesized, how the ABC transporter recognizes the CPS for export, and how is the polymer transported across the two membranes and the periplasmic space.

8 Future Outlook

The cell-bound polysaccharides as discussed in this communication are evolved in the prokaryotes to perform both structural and functional roles in cell growth and division. In this context, in the past few years, the entire genome of the bacteria is available, and a survey of open reading frames indicates that proteins involved in carbohydrate metabolism, carbohydrate-binding proteins, and cell wall assembly proteins constitute the major families of the expressed genes. Therefore, the genes encoding different enzymes or precursors which are responsible during the biosynthetic pathway of these cell-bound polysaccharides in bacteria which act as virulence factors to cause pathogenesis in host cell could be a good target for the development of antibiotic drugs. The structure and function of polysaccharides and the biosynthetic pathways involved in their synthesis would provide an important clue for designing the therapy to combat the infectious diseases caused by the bacteria. Till date, the mechanisms involved in biosynthetic pathways are not completely explored, and certain precursors are still not known thereby it remains a highly challenging problem which could prove to be a promising area for future work.

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