DOI: 10.1002/ejoc.200900682

The Structures of Lipopolysaccharides from Plant-Associated Gram-Negative Bacteria

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Keywords: Lipopolysaccharides / Immunochemistry / Plant-associated bacteria / Glycolipids / Innate immunity / Structure elucidation

Gram-negative bacterial lipopolysaccharides (LPSs) have multiple roles in plant–microbe interactions. LPSs contribute to the low permeabilities of bacterial outer membranes, which act as barriers to protect bacteria from plant-derived antimicrobial substances. Conversely, perception of LPSs by plant cells can lead to the triggering of defence responses or to the priming of the plant to respond more rapidly and/or to a greater degree to subsequent pathogen challenge. LPSs are thus key molecules in the interactions between bacteria and plants, either in symbiosis or pathogenesis. Since LPSs are glycoconjugates genetically and chemically consisting of three different molecular regions, their detailed structure elucidation is a very topical and major scientific task for chemists, and is achieved by a combination of state-of-art chemical and spectroscopic techniques. Knowledge of LPSs' chemical structures is an important prerequisite for any further understanding of the biological processes in plant–microbe interactions. Moreover, the LPSs from Gram-negative bacteria – especially those originating from plant-associated bacteria – are a great source of novel monosaccharides with unusual and occasionally astounding chemical structures, never found in the eukaryotic world. This review presents the structures of LPSs from plant-associated bacteria isolated and identified from 2001 onwards.

Introduction

In an environment that is rich in potentially pathogenic microorganisms, the survival of higher eukaryotic organisms depends on efficient pathogen sensing and rapidly mounted defence responses. The recognition of non-self induces plant defence responses such as the oxidative burst, nitric oxide generation, extracellular pH increase, cell wall strengthening and pathogenesis-related (PR) protein accumulation, leading to basal resistance or innate immunity.[1]

Plants perceive several general elicitors both from host and from non-host pathogens. These elicitors are essential structures for pathogen survival and for this reason are conserved among pathogens. These conserved microbe-specific molecules, also referred to as Microbe- or Pathogen-Associated Molecular Patterns (MAMPs or PAMPs), are recognised as non-self molecules by the plant innate immune systems known as Pattern Recognition Receptors (PRRs).[2] Bacterial lipopolysaccharides have been shown to be a characteristic MAMP recognized in plants.[3–5]

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Lipopolysaccharides (LPSs) are amphiphilic macromolecules present in the outer monolayers of the external membranes of almost all Gram-negative bacteria. They contribute to the structural properties of the cell envelope and play a vital role in bacterial growth, providing a permeable barrier to nutritional substances but a barrier to antimicrobial compounds. In addition, their spatial arrangement with respect to the outer surface of the cell is consistent with their involvement in adhesion, in the mechanism of host recognition, and in the induction of defence-related responses.[6]

The general lipopolysaccharide structure (Figure 1) is common to all Gram-negative bacteria. It is a glycoconjugate, consisting of a lipid moiety called lipid A, anchored to the membrane by fatty acids, that is covalently linked to a saccharide part. This in turn generally consists of two biogenetically different components: an oligosaccharide (core region) and an O-polysaccharide moiety (OPS), the presence or absence of which determines the appearance – smooth or rough – of the bacterial colony. Accordingly, they are named smooth (S-) or rough (R-) LPSs, respectively (R-LPS are also known as LOS, lipooligosaccharides).[6]

The OPS usually consists of a regular polysaccharide with repeating units that rarely contain more than five residues. More than a hundred different monosaccharides, in addition to many types of non-carbohydrate substituents (acyl, amino acid, hydroxy acid, alkoxy and keto acid

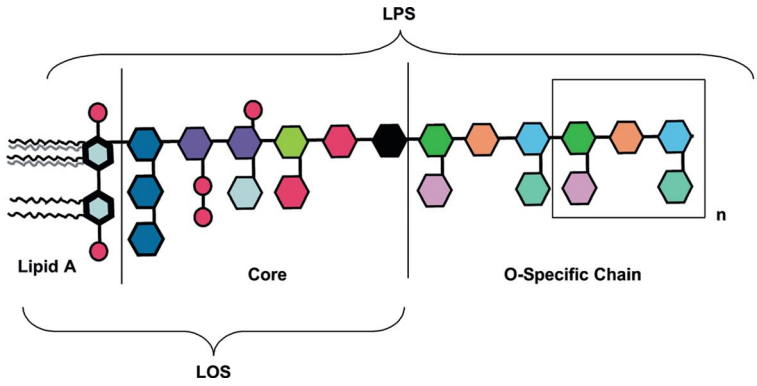


Figure 1. General chemical structure of a LPS from a Gram-negative bacterium. All forms of LPS known to date consist of a lipid A domain and a covalently linked polysaccharide or oligosaccharide portion (LPS or LOS, respectively). The polysaccharide domain is in turn made up of a core region and the O-specific chain. According to the preferential carbohydrate compositions in the core structure, an inner and an outer core region are commonly distinguished. Kdo residues proximal to lipid A are depicted in blue whereas heptose residues are in violet; phosphate residues are also visible as pink appendages.

groups being the most frequent), have so far been found in OPSs, making them the most variable LPS domain. Furthermore, they show size heterogeneity resulting from the number of repeating units, which is responsible for the classical appearance of the SDS-PAGE ladder profiles of LPSs.

Antonio (Tony) Molinaro is Professor of Organic Chemistry and Carbohydrate Chemistry and Biochemistry at the Faculty of Science of the University of Naples Federico II. He took a Masters degree in Biology and then a Ph.D. degree in Chemistry. At the beginning of his career he was interested in the study of structure determination of natural compounds involved in interactions between plants. He then moved to M. Parrilli's group, where he started to study the structures of lipopolysaccharides by a combination of chemical and NMR approaches. He improved his experience in the field of lipopolysaccharides under the direction of Prof. O. Holst, Division of Structural Biochemistry, Borstel Research Center, Germany. He is now responsible for the NMR section of the Interdepartmental Center of Chemical-Physical Methodologies at the University of Naples. He is interested in all aspects of glycosciences and has published over 120 glycomics papers in international peer-reviewed journals of organic and biomolecular chemistry. He is a member of the editorial boards of the following journals: Carbohydrate Research, Marine Drugs, Open Glycoscience and Innate Immunity, the journal of the International Endotoxin Society. He is the chairman of the next 16th European Carbohydrate Congress in 2011 (www.eurocarb2011.org). Apart from this, Tony likes sports and rock music and is a devoted fan of D.A. Maradona and Led Zeppelin.

Mari-Anne Newman took a degree in Biological Science at the University of East Anglia, Norwich UK, then a PhD at the Sainsbury Laboratory, John Innes Centre, Norwich UK, and then a postdoc position in Professor Mike Daniels' Laboratory leading to a permanent position as Associate Professor at the University of Copenhagen, Denmark, in the Department of Plant Biology and Biotechnology. Mari-Anne Newman has always been interested in the mechanisms of plant disease resistance, specifically the understanding of innate immunity in plants. Already as part of her PhD work she was studying the effects of LPSs on plant responses; this work has been continuing during her whole career. In recent years, in a very successful collaboration with Profs. A. Molinaro and M. Parrilli from the University of Naples, Italy, the work has expanded to cover both the chemical and the biological aspects of plant innate immunity.

Rosa Lanzetta graduated in Chemistry at the University of Naples in 1978. Since November 2000 she has been full professor of Organic Chemistry at the Faculty of Science of the University Federico II in Naples. Prof. Lanzetta's main scientific interests lie in structural determination of organic compounds with biological activity. Her scientific research has been particularly directed towards the identification of the structures of complex polysaccharide components of external membranes of Gram-negative bacteria. In particular, she has studied the primary structures of isolated lipopolysaccharides and lipid A components by chemical and spectroscopic methods. The results of these researches have been published in over 150 papers in international peer-reviewed journals of organic chemistry. She is a member of the Società Chimica Italiana, has been coordinator of the Doctorate School in Chemical Science at the University of Naples and responsible for the Interdepartmental Center of Chemical Physical Methodologies, University of Naples.

Michelangelo Parrilli is Full Professor of Organic Chemistry at the Faculty of Science of the University di Napoli Federico II. At the beginning of his career he was interested in the study of reaction mechanisms; his interest then shifted to the field of structure determination of secondary metabolites. For over 25 years he has been working on structure determination of polysaccharides from plants, fungi and, mainly, bacteria. In particular, in this field, he is interested in the structure elucidation of lipopolysaccharide components: O-chain, core oligosaccharide and lipid A. He is also involved in the synthesis of O-chain repeating units in order to investigate structure–activity relationships of lipopolysaccharides from phytopathogenic bacteria. He is the author of more than 200 scientific publications. He is a member of the "Società Chimica Italiana" and of the board of management of the "Gruppo Interdivisionale della Chimica dei Carboidrati". Prof. Parrilli is also a member of the Editorial Board of "Carbohydrate Research".

Less variable is the carbohydrate architecture of the core region, which can be made up of up to 15 monosaccharides, and where both the inner and outer parts are recognized. In particular, only one structural element – the α-linked 3-deoxy-D-*manno*-oct-2-ulopyranosonic acid (Kdo) residue that binds the core region to the lipid A – is basically present in all core regions, although several core regions also possess another characteristic element in the form of L-*glycero*-D-*manno*-heptopyranose (L,D-Hep). Other substituents such as sugars or phosphate residues may be present, as well as acetyl and amino acid residues. In particular LPS core regions, Kdo can be replaced either by its 3-hydroxy derivative, D-*glycero*-D-*talo*-oct-2-ulopyranosonic acid (Ko), or by its 8-amino derivative, 8-amino-3,8-dideoxy-D-*manno*-oct-2-ulopyranosonic acid. In neither case has the biosynthesis or the purpose of the chemical modification of Kdo yet been elucidated.[6]

The lipid A structure consists of a β-(1→6)-linked 2-amino-2-deoxy-D-glucopyranose (D-Glc*p*N, glucosamine) disaccharide that bears (*R*)-3-hydroxy fatty acid residues, two of which are ester-linked to the 3- and 3′-positions, whereas another two are amide-linked at the 2- and 2′-positions [primary fatty acid residues, e.g. (14:0) 3-OH in *E. coli*]. The 3-hydroxy fatty acids are in turn further ester-linked by secondary fatty acids (e.g., 14:0 and 12:0 in LPS of *E. coli*). The hydroxy groups at the 4′-position of the non-reducing GlcN II residue (distal unit) and that of the α-anomeric position of the reducing GlcN I residue (proximal unit) are both substituted with negatively charged groups: mostly phosphate groups (Figure 2).

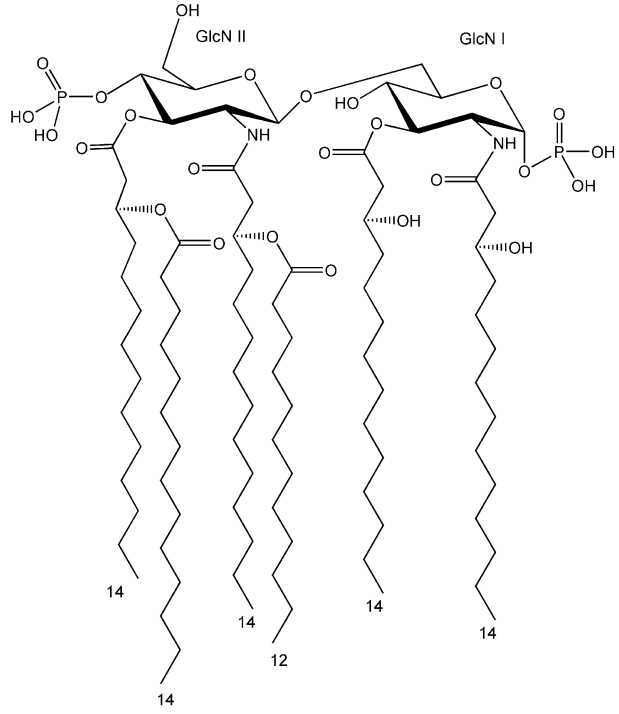


Figure 2. The chemical structure of the lipid A from *Escherichia coli* LPS. The GlcN disaccharide bears primary (*R*)-3-hydroxytetradecanoic acid residues either as ester (at the 3- and 3′-positions) or as amide (at 2- and 2′-positions) systems. The two fatty acid residues on the GlcN II are further esterified by a dodecanoic residue and a tetradecanoic residue. The hydroxy group at the 4′-position in the non-reducing GlcN II residue (distal unit) and that at the anomeric position of the reducing GlcN I residue (proximal unit) are both phosphorylated. At physiological pH the lipid A molecule is negatively charged.

In addition to a general chemical architecture of this form, conserved in many bacterial LPSs, a number of subtle chemical differences responsible for lipid A variation in bacterial LPSs have been identified in the past. Furthermore chemical variations both in mammalian and in plant pathogen bacterial LPSs are responsible for the agonist/antagonist effect of lipid A in host innate immune response processes.[6]

The structures of LPSs from animal-associated bacteria, in particular from the pathogenic bacteria, have been investigated extensively and many excellent reviews on their structures, functions and biosynthesis are available.[7–9] Conversely, the LPSs from plant-associated bacteria have so far been much less intensely studied and characterised, although a review on this topic was published in 2001[10] and recently another review on LPSs from plant pathogenic/symbiotic bacteria was published.[11] Several papers on the biological activity of plant pathogenic LPSs have been published.[3–5,12–16]

The purpose of this review is thus not only to update LPS structural data for plant pathogenic bacteria, but also to extend the investigation to LPSs from nonpathogenic bacteria, such as endophytic and beneficial genera. We do not include the *Agrobacterium* and *Rhizobium* LPS structures because recent reviews have been published.[8,11]

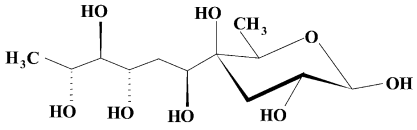
OPS Structures

OPSs belonging to LPSs from the phytopathogens *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Pantoea* are shown in Figure 3.[17–28] Almost every one of these OPSs possesses a backbone of rhamnose residues that can be present in either D or in L configuration and in α or β anomeric configuration; these last can be even found in the same structure. Overall, beside rhamnose, the repeating units show a very limited variability of residues and are often linear or bear a single branching monosaccharide. In particular, in the case of *Pseudomonas syringae* (*Ps*), GlcNAc and Fuc3NAc can be found attached to the rhamnan chain[17–19] whereas in *Xanthomonas* OPS, Xyl and Fuc were also found.[21,23–26] More variability occurs in *Erwinia* LPSs, in which Gal, Glc and Man can be found either in the main skeleton or in the side arms.[26–27] In addition, in *Erwinia carotovora* ssp. *atroseptica* (*Eca*) OPS a ten-carbon branched sugar – the 3,6,8-trideoxy-4-*C*-[(*R*)-1-hydroxyethyl]decose, common name erwiniose[28] – was found. Interestingly, this monosaccharide is structurally very similar to caryophyllose, a twelve-carbon branched sugar found in the OPS of *Burkholderia caryophylli*, another plant pathogenic bacterium, responsible for the wilt disease in carnation (Figure 4).[29–30]

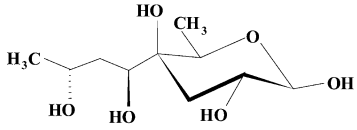
The OPS structures of endophytic *Azospirillum* and *Burkholderia* LPS and mushroom pathogenic *Pseudomonas* LPSs are reported in Figure 5.[31–39]

Species	Structure	Ref.
Pseudomonas syringae ***pv. porri*** **NCPPB 3365** [a]		
Major component	β-GlcNAc (1 → 2) →3)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	[17]
Minor component	β-GlcNAc (1 → 2) →2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	
Minor component	→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	
Pseudomonas syringae **pv.** ***mori***	→2)-β-L-Rha-(1→	[18]
Pseudomonas syringae ***pv. coronafaciens*** **IMV 9030**		
Major component	→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	[19]
Minor component	→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	
Minor component	α-D-Fuc3NAc (1 → 3) →2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	
Pseudomonas syringae ***pv. atrofaciens*** **IMV 8281**	α-L-Fuc3NAc (1 → 2) →2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	[19]
	α-L-Fuc3NAc (1 → 2) →2)-α-L-Rha-(1→2,3)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→	
Pseudomonas cichorii **5707**	→3)-α-L-FucNAc-(1→3)-α-D-QuiNAc-(1→3)-α-L-FucNAc-(1→2)-β-D-QuiNAc-(1→	[20]
Xanthomonas campestris ***pv. malvacearum*** **GSPB 1386** [b]	α-D-Fuc*f* (1 → 4) →3)-α-D-Rha-(1→3)-α-D-Rha-(1→2)-α-D-Rha2Me-(1→	[21]
Xanthomonas campestris ***pv. phaseoli fuscans*** **GSPB 271**	→3)-α-D-Rha-(1→3)-α-D-Rha-(1→2)-α-D-Rha-(1→	[21]
Xanthomonas cassavae	β-L-Xyl (1 → 2) →3)-β-D-Rha-(1→3)-α-D-Rha4NAc-(1→	[22]
Xanthomonas campestris vitians [c]	[–3)-α-L-Rha-(1→]$_n$3)-β-L-Rha-(1– α-D- Fuc3NAc- (1 (2 ↑) *n* = 1,2,3	[23]
Xanthomonas campestris 8004	–3)-α-D-Rha-(1→3)-β-D-Rha-(1– α-D- Fuc3NAc-(1 (2 ↑)	[24]
Xanthomonas campestris ***pv. pruni*** [d]	β-D-Xyl (1 → 4), β-D-Xyl (1 → 4), β-D-Xyl (1 → 4) →2)-α-L-Rha-(1→2)-α-D-Glc-(1→3)-α-L-Rha-(1→	[25]
Erwinia carotovora ***ssp. carotovora*** **GSPB 436**	α-D-Glc (1 → 3) →3)-β-L-Rha-(1→4)-α-L-Rha-(1→3)-α-D-Fuc-(1→	[26]
Erwinia carotovora ***ssp. atroseptica*** [e]	β-Erw-(1→3)- α-D-Gal2Ac (1 → 2) →3)-α-D-Gal-(1→3)-α-D-Man-(1→4)-α-L-Rha-(1→	[27]
Pantoea agglomerans **FL1**	→2)-α-D-Rha-(1→2)-α-D-Rha-(1→3)-α-D-Rha-(1→3)-α-D-Rha-(1→	[28]

Figure 3. The OPSs belonging to LPSs from the phytopathogens *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Pantoea* are shown. Residues are in pyranose form if not stated otherwise. [a] In strain NCPPB 3364 the same three OPSs are present but the major component is the second one. [b] A methyl group at O-3 is present in around 65% amount. [c] Fuc3NAc is nonstoichiometric, and when present has no fixed α-Rha to substitute. The rhamnan backbone is more frequently a trisaccharide; *n* is more frequently equal to 2 but it can also assume values of 1 and 3. [d] Xylose units are present in nonstoichiometric fashion. [e] Erw is erwiniose (see text and also Figure 4).



Caryophyllose



Erwiniose

Figure 4. The chemical structures of erwinose and caryophyllose residues.

Endophytes are able to colonize intercellular spaces and vascular tissues of plants without causing plant diseases. Strains of *B. cepacia* can grow as endophytes, and other soil species also play an important role as biocontrol and plant growth promoting agents in the rhizospheres of several crop plants. *B. cepacia* is able to colonize the surfaces of hyphae of *Fusarium oxysporum* sp. *cubense* and the fungal macrospores. In vitro colonization of the fungi by the endophyte results in mycelial deformation with terminal and intercalary swelling. *B. phytofirmans* strain PsJN is capable of endophytic colonization of potato, grapevine, tomato and other plants and can promote plant growth and health. Grapevine plantlets co-cultured with PsJN grew more quickly and possessed significantly more secondary roots;

Species	Structure	Ref.
***Burkholderia phytofirmans* PsJN** [a]	→3)-α-L-6dTal-(1→3)-β-D-GalNAc(1→ 2,4 ↑ β-D-Xyl-(1	[31]
***Burkholderia cepacia* ASP B 2D**	→2)-β-D-Rib-(1→6)-α-D-Glc(1→	[32]
***Azospirillum brasilienses* SP245**	→2)-β-D-Rha-(1→3)-α-D-Rha-(1→3)-α-D-Rha-(1→2)-α-D-Rha-(1→2)-α-D-Rha-(1→	[33]
***Azospirillum lipoferum* Sp59B**	α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-β-D-Man 4 ↑ –3)-β-D-Gal-(1→3)-α-D-Gal-(1	[34]
***Azospirillum brasilienses* S17** [b]	→ 4)-α-L-Rha2Me-(1→3)-α-D-ManN*S*Hb-(1– 4 ↑ β-D-GlcNAc-(1 -D-Glc 1 ↓ 2 → 3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha(1→	[35]
***Azospirillum lipoferum* SR65**	-D-Glc 1 ↓ 3 → 2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha(1→	[36]
***Azospirillum irakense* KBC1**	α-D-Gal*f*-(1→2)-α-L-Rha-(1→3)-β-D-Man-(1→3)-α-L-Rha 1 ↓ 3 –4)-α-L-Rha-(1→3)-β-D-Gal-(1	[37]
Pseudomonas reactans [c]	→ 3)-β-D-QuiNAlaAc4NAlaAc-(1→3)-α-D-Glc2Am-(1→3)-α-D-QuiNAc4NAc -(1→	[38]
Pseudomonas tolaasii	→ 4)-α-L-GulNAc3AcAN-(1→3)-β-D-QuiNAc-(1→4)-α-L-GulNAcAN-(1→3)-β-D-QuiNAc-(1→	[39]

Figure 5. The OPSs belonging to LPSs from *Azospirillum* and *Burkholderia* and mushroom pathogenic *Pseudomonas* LPSs. Residues are in pyranose form if not stated otherwise. [a] Xylose units are present in nonstoichiometric fashion, either substituting O-2 or O-4; 6dTal is 6-deoxytalose. [b] *S*Hb is 3-hydroxy butyric acid. [c] Am is acetamidino.

PsJN also enhances the resistance of tissue culture plantlets to fungal disease and heat stress and protects grapevines against *Botrytis cinerea*.

The mechanism of this protection is not localized but systemic; this phenomenon is known as rhizobacteria-mediated induced systemic resistance (ISR) and is one mode of action of disease suppression by non-pathogenic rhizosphere bacteria. Host defence response pathways are primed by the colonizing beneficial bacteria, allowing a much faster response to pathogen infection [i.e., formation of structural barriers, such as thickened cell wall papillae due to the deposition of callose (a β-1,3-glucan) and the accumulation of phenolic compounds at the site of pathogen attack].[40]

Azospirillum is a bacterium associated with the roots of monocots, including important crops such as wheat, corn and rice, a number of strains of which have been shown to exert beneficial effects. *Azospirillum* can establish an associative symbiosis with cereals, but unlike in the case of mutualistic symbiosis the association is not accompanied by formation of new organs, such as in the interaction of *Rhizobium* and leguminous plants.

All plant-associated bacteria of this kind thus qualify as Plant Growth Promoting Rhizobacteria (PGPRs). The molecular mechanisms for these phenomena are still under investigation, but LPSs play a pivotal role in the interactions of plants with Gram-negative endophytes.

The monosaccharide compositions of Gram-negative endophyte LPSs resemble those of phytopathogenic OPSs, although there is a clear prevalence of 6-deoxyhexose monosaccharides, essentially fucose and 6-deoxytalose, but not rhamnose as in the OPSs from phytopathogenic bacteria.

In summary, the monosaccharide compositions of the OPSs of plant-associated bacteria determined until now seem to show some common characteristics: rather limited monosaccharide variability with predominance of 6-deoxyhexose residues and amino sugars, whereas acid residues are completely missing. In animal pathogenic bacterial LPSs, the OPS represents the antigenic moiety (i.e., it is this LPS moiety involved in the acquired immunity recognition mechanisms). Consequently, whereas animal pathogenic bacteria show really large numbers and combinations of monosaccharides, much lower structural variability is exhibited by plant-associated OPSs; this in part reflects much weaker pressure to change or alter their epitopes, because plants do not possess acquired immune systems.

P. reactans and *P. tolaasii* are two saprophytic bacteria associated with cultivated mushrooms, and their OPS structures show monosaccharide compositions rather different from those arising from plant-associated bacteria, which could be ascribed to the different biochemical milieu that both *P. reactans* and *P. tolaasii* have to face (mushrooms are very different from plants).[38–39] In particular, their LPSs do not contain any 6-deoxyhexose residues but have 6-deoxyhexosamine almost exclusively, and even in this case uronic acids are lacking or, when present, the carboxyl group is protected as an amide.

Core Structures

As far as the structures of the core oligosaccharides of phytobacteria are concerned, data have only recently been added to the literature.[11] Chemical variation in core region structures has a more limited range than in O-specific polysaccharides. Only one structural element is present in all core regions; this is the α-linked Kdo residue that binds the core region to the lipid A. Several core regions also possess L,D-Hep and the basic oligosaccharide skeleton oligosaccharide L,D-Hep-(1→7)-L,D-Hep-(1→3)-L-α-D-Hep-(1→5)-[α-Kdo-(2→4)]-α-Kdo, with further glycosyl substitutions on this backbone furnishing structural variability. Other substituents may be sugars or phosphate and acetyl groups, and amino acid residues may also be present. Several LPSs lack heptose residues completely. Both Kdo residues can be replaced by the stereochemically similar sugar Ko (in *Burkholderia*, for example).

The core oligosaccharide structure of *Ps* LPSs contains several of the canonical features described above, together with two highly phosphorylated glycoforms terminating either with L-rhamnose or with Kdo.[41] In contrast, the *P. cichorii* LPS core region does not contain any phosphate group or heptose residue, but has a *galacto*-configured residue directly bound to Kdo (Figure 6).[42]

In the case of *Xanthomomnas campestris* (*Xc*) the inner core region, as in many LPSs, carries anionic substituents, which in this case are located on Kdo and α-mannose residues; the Kdo residue carries an α-galacturonyl-phosphate substituent at its O-4 position whereas the adjacent α-mannose is substituted at O-3 by a second α-galacturonyl phosphate or, alternatively, by a phosphoramide group (Figure 6).[4,14] All of these chemical groups are great structural novelties for the core oligosaccharide regions of LPSs and were found for the first time in *Xanthomonas*. However, the presence of negatively charged substituents in close proximity to the lipid A core region is functionally important for intermolecular associations by cross-linking of divalent cations, and it is thought that this electrostatic interaction contributes to enhance the stability of the external bacterial membrane with the formation of a strong, rigid and protective barrier.[4]

Lipid A

Generally, lipid A is the less variable component of LPSs in terms of chemical architecture, and the main differences seen in lipid A are mainly in the acylation pattern, according to the numbers, locations and lengths of fatty acids. In all LPSs, the lipid A is a mixture of an intrinsically heterogeneous blend of molecules ranging from under-acylated (three or four fatty acids) to over-acylated (seven fatty acids) lipid A species.[6] The prevalence of one species over another is of particular importance in view of the fact that fatty acid composition and distribution strongly affects lipid A/LPS endotoxic activity in hosts, both in animals and

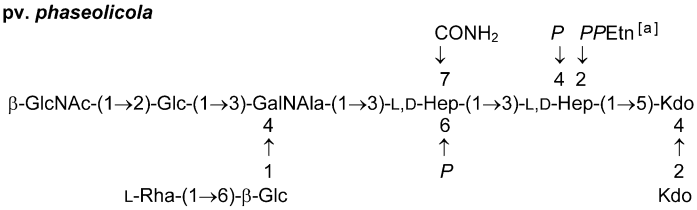
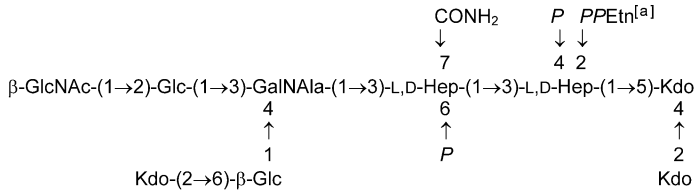
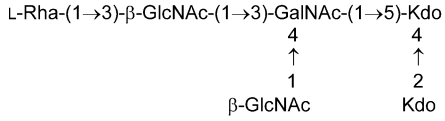
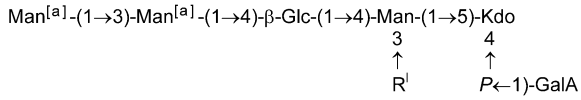
Species	Structure	Reference
Pseudomonas syringae* pv. *phaseolicola		
Glycoform 1		[41]
Glycoform 2		
Pseudomonas cichorii		[42]
Xanthomonas campestris* pv. *campestris		[4]

Figure 6. The core oligosaccharide regions of LPSs from *P. syringae* and *X. campestris*. Residues are in α-D configuration and pyranose form if not stated otherwise. [a] Nonstoichiometric substitution; R, phosphoramide or GalA-1*P*.

plants. The lipid A variants from *Erwinia* and *Pseudomonas* both possess the typical bis-phosphorylated disaccharide backbone, to which seven or six fatty acids are attached (Figure 7).[10] No other polar heads group are present, whereas in *B. cepacia* (A. Molinaro, unpublished result) and *B. caryophylli* a phosphoryl-arabinosamine group can occur either at the anomeric position or at the C-4 position of GlcN II.[43]

Species	GlcN II substitution			GlcN I substitution			Ref.
	O-4'	O-3'	N-2'	O-3	N-2	O-1	
Erwinia carotovora	P	14:0 (3-OH) 12:0	14:0 (3-OH) 12:0	14:0 (3-OH)	14:0 (3-OH) 16:0	P	[10]
Agrobacterium tumefaciens	P	14:0 (3-OH)	16:0(3-O) (28:0 27-O) 4:0 3-OH [a]	14:0 (3-OH)	16:0 (3-OH)	P	[41]
Azospirillum lipoferum	–	14:0 (3-OH)	16:0 (3-OH) 18:1/ 18:0	14:0 (3-OH)	16:0 (3-OH)	GalA	[44]
Burkholderia cepacia *Burkholderia caryophylli*	Ara4N-P	14:0 (3-OH)	16:0 (3-O) 14:0	14:0 (3-OH)	16:0 (3-OH)	Ara4N-P	[43]
Xanthomonas campestris [a]	P-P-EtN	12:0 (3-OH) [a] 10:0 [a]	12:0 (3-OH) [a]	12:0 (3-OH) [a] 10:0 [a]	12:0 (3-OH) [a]	P-P-EtN [b]	[45-46]
Pseudomonas cichorii *Pseudomonas corrugata*	P	10:0 (3-OH)	12:0 (3-OH) 12:0	10:0 (3-OH)	12:0 (3-OH) 12:0	P	[10, 42]

Figure 7. Substitution patterns on the two GlcN residues on different plant pathogenic bacterial lipid A. [a] High structural heterogeneity in the lengths of primary and secondary fatty acids, from 10:0 (3-OH) to 13:0 (3-OH) and10:0 to 13:0, respectively. [b] Present in nonstoichiometric amount.

An exception is found in the lipid A of *Azospirillum lipoferum*, in which the only negative charge in the lipid A is represented by a galacturonic acid that replaces the phosphate group at an anomeric position.[44]

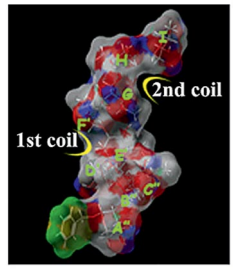
The lipid A of *Xanthomonas* exists as a mixture of hexacylated species in which there is a remarkable heterogeneity in fatty acid length, which varies from 10 to 13 carbon atoms.[45] Interestingly, the non-pathogenic mutant strain 8530 of *Xc* pv. *campestris* (*Xcc*) produces a penta-acylated lipid A, lacking a secondary fatty acid, rendering strain 8530 lipid A inactive in elicitation of innate immunity responses.[46] This suggests that *Xanthomonas* has the capacity to modify lipid A to affect its activity. Such effects may occur in wild-type bacteria exposed to stresses such as those that may be encountered during plant colonization and disease.

The Roles of LPSs in Plant Innate Immunity

LPSs have been shown to have multiple roles in plant–microbe interactions. LPSs contribute to the restrictive Gram-negative membrane permeability, allowing bacterial growth in unfavourable environments. LPSs and their derivatives act as MAMPs and induce innate immune responses in plants. Earlier studies in plants have shown that LPSs can prevent the hypersensitive response (HR) induced by avirulent bacteria. The HR, which is characterised by localised cell necrosis at the infection site, is the triggering of gene-for-gene resistance in plants caused by invading pathogens carrying effectors that are specifically recognised by corresponding Resistance gene (R-gene) products in the plant.

As stated above, the O-antigens of the LPSs from many phytopathogenic bacteria have been shown to consist of oligorhamnans. In order to further knowledge of the structural epitopes within LPS that trigger immune responses in plants, synthetic O-antigen polysaccharides, oligorhamnans of increasing chain lengths, were tested in the model plant *Arabidopsis thaliana* cv. Colombia (*Arabidopsis*). Tri-, hexa- and nonasaccharides were chemically synthesized and were found to suppress the HR, as well as to act as MAMPs and to elicit transcription of the pathogenesis-related genes *PR1* in *Arabidopsis*.[3] The efficiency of HR suppression and *PR* gene induction improved with increasing sugar chain length in the synthetic O-antigens. In addition, a coiled structure was observed with increasing chain length, indicating a role for this structure as a MAMP and by correlation a role for the O-antigen from phytopathogenic bacteria in plant innate immunity (Figure 8).

To study whether the mammalian innate immune system has parallels in the plant system, the roles and mechanisms of action of LPSs and their derivatives, the core oligosaccharides and the lipid A moieties in plant–bacteria interactions were investigated in the plant model *Arabidopsis*. R-LPS was found to induce the defence-related *PR1* and *PR2* genes in two temporal phases in *Arabidopsis*: the core oligosaccharide induced only the early phase and the lipid A moiety only the later phase, which suggests that both the



C

Time after treatment	Nonasaccharide	Water
0 hours	No transcript	No transcript
12 hours	+52.6	No transcript
20 hours	+86.8	No transcript
24 hours	+26.9	No transcript

Figure 8. A) Connelly surface model of a synthetic oligorhamnan nonasaccharide. B) HR suppression by nonnasaccharides in *Arabidopsis thaliana*. The leaf on the left was pre-treated with nonasaccharides (50 $\mu g\,mL^{-1}$) followed 20 h later by *Pseudomonas syringae* pv. *tomato* (*Pst*) *AvrRPM1* (10^7 cfu mL^{-1}); the leaf on the right was pretreated with H_2O followed by *Pst AvrRPM1*. Twenty hours later complete abolition of the hypersensitive response (HR) was seen in the nonasaccharide pre-treated leaf, whereas the leaf pretreated with water developed HR in response to *Pst AvrRPM1*. C) The ability of nonasaccharides (50 $\mu g\,mL^{-1}$) to induce *PR-1* gene expression in *Arabidopsis thaliana*. +) Fold upregulation of *PR-1* transcript relative to water-treated tissue, after normalisation to *18SrRNA* $p<0.001$.

core oligosaccharide and the lipid A are recognised by plant cells (i.e., both act as elicitors). These findings provide support for roles of *Xcc* lipid A and the *Xcc* core oligosaccharide as MAMPs of innate immunity in plants. We speculated that the different LPS fragments are recognised by different plant receptors.[4] A study of global transcriptional changes in *Arabidopsis* cells treated either with *B. cepacia* LPS (100 $\mu g\,mL^{-1}$) or *P. syringae* hairpin (50 $\mu g\,mL^{-1}$) revealed, surprisingly, that both LPS and hairpin induced only very few *PR* transcripts, indicating that this cell culture system responds differently from a whole plant to these bacterial elicitors .[47]

Determination of the structure of lipid A from *Halomonas magadiensis* (*H. magadiensis*), a Gram-negative extremophilic and alkaliphilic bacteria isolated from a soda lake in the East African Rift Valley, suggested that the molecule might act as a LPS antagonist in human cells.[48] *H. magadiensis* lipid A, characterized by an unusual and very low degree of acylation, was verified to inhibit *Escherichia coli* lipid A-induced immune responses in human cells. Consistently with the ability in blocking enteric

LPS-induced human monocyte activation, we found that *H. magadiensis* lipid A was able to antagonise the action of *E. coli* lipid A when inducing *PR1* gene expression in *Arabidopsis*.[49] Even though the mode of perception of LPS in plants is far less understood than in mammals and insects, these results indicate that *Arabidopsis* is sensitive to the same lipid A structures that determine biological activity in humans.[49,50]

In correlation with studies in mammalian systems, in which it is well established that the phosphorylation pattern of lipid A affects its biological activity,[50] dephosphorylated *Xcc* R-LPS was not recognised in plants.[4] LPS has been found not only to induce defence responses, but also to prime expression of plant defence responses upon subsequent bacterial inoculation [e.g., to promote an early triggering of the synthesis of the antimicrobial compounds feruloyl tyramine (FT) and *p*-coumaroyl tyramine (CT) in pepper plants].[13,40,51]

In *Arabidopsis* the O-antigen part of the LPS molecule is thought to be responsible for ISR. Early studies showed that LPS from the rhizobacteria *P. fluorescens*, as well as the live bacteria, induced ISR in carnation and radish, whereas mutant bacteria lacking the O-antigen side chain could not induce ISR.[52–53]

In contrast with the rhizobacteria-mediated ISR, systemic activation of defence-related responses in plants upon local necrotizing pathogen infection is referred to as systemic acquired resistance (SAR). SAR is accompanied by a systemic increase in salicylic acid (SA), and SA is required for SAR signalling.[54–55] However, recent studies suggest that recognition of the MAMPs, LPS or flagellin, and not formation of necrotic lesions, contributes to the bacterial induction of SAR in *Arabidopsis*. Treatment of *Arabidopsis* with *P. aeruginosa* LPS, flagellin or non-host bacteria was shown to be associated with accumulation of SA, expression of the *PR* genes and expression of the SAR marker gene *Flavin-dependent monooxygenase 1* in both treated and distant leaves.[56–57]

Alterations in lipid A or other structures within LPSs are known to occur during symbiotic interactions with plants[58] and in response to compounds in plant root exudates[59] and may occur during plant pathogenesis. These alterations may serve both to increase the resistance of the bacteria against host defences and to attenuate the activity of lipid A or LPS in triggering those defences. Studies of the R-LPS of a mutant of *Xcc* defective in core completion have shown that loss of the glycosyl residues of the outer core is accompanied by alterations in the lipid A, which becomes penta-acylated (rather than hexa-acylated) and is substituted with phosphoethanolamine moieties.[46] This modified lipid A is not able to induce defence-related responses such as *PR* gene induction in *Arabidopsis*. This suggests that *Xcc* has the capacity to modify the structure of the lipid A in order to reduce its activity as a MAMP. Whether alterations in the acylation pattern or phosphoethanolamine substitution of *Xcc* lipid A occur in the host and whether they are triggered by specific plant environmental cues is as yet unknown.

Until recently, the activity of LPSs in plants has only been described in dicots, but current studies in rice cells have revealed that LPSs from various pathogenic and non-pathogenic bacteria induce the generation of reactive oxygen species and defence-related gene expression in monocots, indicating that the machinery recognising LPS is evolutionarily conserved in monocots and dicots.[60]

Discussion

The mechanisms by which LPSs are perceived by plants are still not understood. The concentrations of LPSs required to elicit most of the effects described above are in the 5–100 $\mu g\,mL^{-1}$ range, which suggests that plants do not have the exquisite sensitivity to LPS shown by mammalian cells, which can respond at concentrations in the $pg\,mL^{-1}$ to $ng\,mL^{-1}$ range. These considerations have led to suggestions that plants possess only low-affinity systems to detect LPSs,[61] although plants can detect other bacterial MAMPs such as peptides derived from flagellin and Ef-Tu elongation factor at $pg\,mL^{-1}$ levels. One complicating factor is the aggregation of LPS molecules within the purified preparations used, which could affect the abilities of LPSs to traverse the matrixes of plant cell walls to reach presumed membrane-associated receptors.

The unravelling of the components involved in plant innate immunity is important for understanding of the complex interactions between plants and bacterial pathogens, and perhaps between bacterial pathogens and mammals. The similarities of the innate immune systems in plants and mammals, and the microbial capacity for cross-kingdom pathogenicity of human pathogens, has led a few researchers to use the *Arabidopsis* model plant to study human microbial pathogenicity factors.[62]

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Received: June 19, 2009
Published Online: September 29, 2009

