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Review

# Lipopolysaccharide structures from *Agrobacterium* and *Rhizobiaceae* species

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**Abstract**—This review reports and discusses the structural and the biological data available for the lipopolysaccharides from the Gram-negative bacterium *Agrobacterium* together with those of other related *Rhizobiaceae* species.


*Keywords:* *Agrobacterium*; *Rhizobium*; Lipopolysaccharide; Tumor induction; Symbiosis

**Contents**



## 1. Introduction

Lipopolysaccharides (LPSs) constitute one of the main components of the outer leaflet of almost all Gram-negative bacteria where they play an essential role for bacterial growth and survival.[1] In particular, their external location enables them to communicate with the environment which, in the case of plant bacteria, is mainly constituted by a plant host. The main roles played by LPSs in the bacteria–plant interaction are to provide a structural barrier to plant-derived antimicrobial compounds, plant recognition, plant adhesion, induction of defence-related responses and infection and nodule invasion in plant symbiosis.

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The general structural design of these glycoconjugate macromolecules is the same in Gram-negative genera and consists of two chemically different moieties: a glycolipid part, named lipid A, which is anchored into the outer membrane, and a saccharide part, which is oriented towards the outside of the cell. The latter moiety can be further divided into two regions: an oligosaccharide part, named core, and an O-specific polysaccharide (OPS), which is responsible for the antigenic property of LPSs. The core oligosaccharide, which may be subdivided into an inner and outer core, constitutes the covalent bridge between the lipid A and the OPS. The presence or the absence of the OPS defines a smooth or rough LPSs (named also lipooligosaccharide, LOS) in agreement with the smooth or rough appearance of bacterial colonies, respectively.

When, some few years ago, we published a review[2] on the structures of the LPSs of Gram-negative phytopathogenic bacteria we noted that, besides investigations on the most common *Pseudomonas*, *Burkholderia*, *Ralstonia*, *Xanthomonas* and *Erwinia* species, structures of the LPSs of *Agrobacterium* species had never been described. This prompted us to investigate *Agrobacterium* species collecting, within a few years, several structures of their LPSs.

This review reports the updated structural data of *Agrobacterium* LPSs and compares it with that of LPSs from *Rhizobium* species. These two genera belong to the *Rhizobiaceae* family and their names will be maintained throughout the discussion, although a recent taxonomical proposal wants to merge the *Agrobacterium* genus with *Rhizobium*.[3]

Both genera generally share the same habitat, the soil, but the outcome of interaction with the plants is rather different. Bacteria of the genus *Rhizobium* (and related genera as *Sinorhizobium*, *Mesorhizobium*, and *Bradyrhizobium*) are plant symbionts and each species is able to select a particular legume plant host. The interaction with the roots leads to a beneficial bacteria–plant interaction accomplished from the formation of root nodules where free-living bacteria differentiate into intracellular bacteroids which fix the atmospheric nitrogen and produce nutrients for the host.

*Agrobacterium* species are phytopathogenic, with either tumor-inducing or hairy-roots tumor inducing ability, with *A. radiobacter* as the only exception being not phytopathogenic at all (but also not symbiotic) and currently used as a biocontrol agent in the protection against *A. tumefaciens*.[4]

The genus *Agrobacterium* currently comprises five pathogenic species: *A. tumefaciens* harboring the Ti plasmid and able to induce the crown gall disease, *A. rubi* with pathogenic features similar to *A. tumefaciens* but interacting preferentially with *Rubus* plants, *A. vitis* producing crown gall disease exclusively on *Vitis vinifera*, *A. rhizogenes*, harboring the Ri plasmid and inducing the hairy-roots phenotype, and *A. larrymoorei*, isolated in 1991 from tumors on *Ficus benjamina*.[5] For the most studied member of this genus, *A. tumefaciens*, the pathogenesis mechanism is well established. Each strain of this Gram-negative bacterium induces the crown gall disease in a wide range of dicotyledonous plants, and in particular in the members of *Rosaceae* such as apple, pear and cherry. The disease gains its name from the large tumor-like swellings (galls) that typically occur at the crown of the plant, just above soil level. The growth of all these plants is compromised, leading to damages to nursery stocks and to their marketability. This disease is one of the most widely studied because of its remarkable biology; basically, the bacterium transfers the t-DNA, a portion of its plasmidial DNA (called Ti, i.e., tumor inducing), into the plant host genome, where it is integrated, causing the uncontrolled growth of the modified plant cells and then the formation of the tumor. This unique mode of action of *A. tumefaciens* made the bacterium applicable as tool to produce transgenic plants.

The development of the disease is a complex process and is conditioned by the recognition and adsorption of the bacterium on the host: according to the accepted mechanism, *A. tumefaciens* is attracted to wound sites of the root surfaces by chemotaxis, and the presence of phenolic compounds, as acetosyringone, in synergy with a certain class of monosaccharides (D-glucose, D-galactose, L-arabinose) triggers the activation of the virulence genes.[6] To transfer its t-DNA into the plant cell, the bacterium has to be adsorbed on the wounded area which is modulated by the components of the external membrane of the bacterium, both the proteins and the LPSs.[7] The importance of these molecules in the adhesion process is demonstrated by the reduced virulence displayed by mutants with defective or altered O-antigenic moiety compared to the wild type.[8]

Both *Agrobacterium* and *Rhizobium* comprise different species that can naturally present S- or R-type LPS, consequently, the structural data are grouped according to the presence of the OPS.

## 2. LPS structure

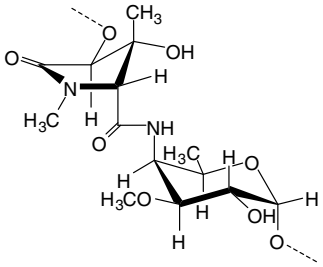
### 2.1. OPS

The OPS represents the most variable domain of LPSs and is generally constituted by a regular polysaccharide with repeating units usually consisting of one to five residues with a very large monosaccharide variety giving rise to an enormous structural diversity, which can be further increased by several types of substituents (decorations). In addition, the OPS is characterized by a size heterogeneity due to the number of repeating units, up

to 40, which leads to the ladder profile of LPS on SDS-PAGE. In the case of pathogenic bacteria, several investigations have indicated that the composition or the size of the OPS can modulate the virulence potential of the bacterium. In the case of symbiotic bacteria, the OPS structure seems to be involved in the steps of infection process, including colonization (adhesion) and the ability to bypass or overcome host defence mechanisms. In Tables 1 and 2, the repeating units of the OPS of *Agrobacterium* and *Rhizobium* smooth-form LPSs (wild type strains) are listed.

A comparison of the monosaccharide compositions of the OPS for both genera reveals a prevalence for 6-deoxy-sugars, in particular rhamnose, fucose and 6-deoxy-talose. These sugars do not appear to be correlated to virulence because they occur in all species, regardless of their pathogenic or symbiotic activity. Furthermore, it is interesting to note that the dominance of rhamnose is shared by other phytopathogenic bacteria[2] which, on the contrary, contain much less frequently fucose or 6-deoxy-talose. In particular, the last monosaccharide is rather rare, whereas fucose appears only as terminal side chain and not in the chain backbone as in *Agrobacterium* and *Rhizobium* LPSs. Thus, Fuc and 6d-Tal represent a particular feature of *Rhizobiaceae* members.

**Table 1.** OPS structures from LPS of *Agrobacterium* species

| Species | Collection | Structure | Ref. |
|---|---|---|---|
| *A. tumefaciens* B6 | DSM 30205 | 3)-α-D-Ara*f*-(1→3)-α-L-Fuc-(1→ | 43 |
| *A. tumefaciens* C58 | DSM 5178 | 3)-α-L-4OAc6dTal-(1→Acetyl substitution is not stoichiometric | 9 |
| *A. tumefaciens* F/1 | DSM 30206 | 3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→<br>4)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→ | 44 |
| *A. tumefaciens* TT9 | DSM 30208 | 4)-α-L-Rha-(1→3)-α-D-Fuc-(1→ and  | 45 |
| *A. radiobacter* M2/1 | DSM 30199 | 3)-α-L,D-Hep-(1→ | 46 |
| *A. radiobacter* | DSM 30147 | 2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→<br>3)-α-D-Fuc-(1→3)-α-D-Fuc-(1→<br>2<br>α-D-Man-1┘ | 47 |

All monosaccharides are pyranoses, except where specified.

**Table 2.** OPS structures from LPSs of *Rhizobium* species and related genera

| Species | Structure | Ref. |
|---|---|---|
| *R. leguminosarum* bv. *trifolii* 4S | 3)-α-L-Rha-(1→3)-α-L-Rha-(1→4)-β-D-GlcNAc-(1→3)-α-L-Rha-(1→<br>2<br>α-D-ManNAc-1┘ | 48 |
| *R. etli* CE3 | 4)-β-D-6OMeGlcA-(1→4)-α-L-Fuc-(1→<br>3<br>α-3OMe6dTal-1┘ | 49 |
| *R. tropici* CIAT899 | 4)-β-D-Glc-(1→3)-α-D-2OAc6dTal-(1→3)-α-L-Fuc-(1→ | 50 |
| *R. loti* NZP2213 | 3)-α-L-2OAc6dTal-(1→ | 10 |
| *R. leguminarum* bv. *viciae* 128C53 | 3)-α-L-Rha-(1→3)-α-L-Fuc-(1→3)-α-L-Fuc-(1→<br>2<br>α-D-Man-1┘ | 16 |
| *Mesorhizobium huakuii* IFO15243T | 2)-α-L-Rha-(1→2)-α-L-6dTal-(1→3)-α-L-6dTal-(→ | 51 |

All monosaccharides are pyranoses, except where specified.

Further structural similarities are found in LPSs of *A. tumefaciens* strain C58[9] and *Rhizobium loti* NZP2213,[10] which share the same α-L-6d-talan backbone, but with a different acetylation pattern, being non-stoichiometric at O-4 in *Agrobacterium* C58, and stoichiometric at O-2 in *R. loti* NZP2213.

### 2.2. Core oligosaccharide structures

The core region consists of an oligosaccharide with up to 15 residues, located between the lipid A and, in S-form LPSs, the OPS. Considering the most investigated *Enterobacteriaceae* LPS, the core contains *inter alia* heptoses and 3-deoxy-D-*manno*-oct-2-ulopyranosonic acid (Kdo), which is always present and links the core region to the lipid A moiety. The structural variability is mainly due to other sugar substituents and decorations, which often are constituted by phosphate groups. The possibility to investigate the core structure in wild type strains depends on the OPS length—it is generally low in the case of S-form LPSs, due to the predominance of the OPS components, whereas it is more immediate in the case of R-form LPSs (LOS). Thus, in the case of S-form LPSs it is necessary to resort to an OPS defective mutant strain or to degrade selectively the OPS. The role of core oligosaccharide in LOS seems to be that of the OPS in LPSs, that is, it is involved in antigenic and host-adhesion mechanisms. In addition, due to its less structural variability, particularly in its inner part, the core region may play a taxonomical role too.

Tables 3 and 4 report the core structures known for LPSs of *Agrobacterium* and *Rhizobium*. Presently, several core structures are reported for *Agrobacterium* LPSs, whereas in the case of the other *Rhizobaceae* most of the structures are incomplete. However, the LOS of *Rhizobium etli* CE3[11] is an exception since core structure and its biosynthesis are reported.[12–15] The structure is identical to *R. leguminosarum* bv. *viciae* LOS, and similar to *R. leguminosarum* bv. *trifolii* and of *R. etli* CE3 LOSs.[16,17]

Interestingly, all *Agrobacterium* and *Rhizobium* LOS core regions differ from most of LOS by the lack of heptoses and phosphate groups and these similarities confirm the close taxonomical relationship between these two genera. In this regard, we have focused our attention on the substituents of the inner Kdo residue which

**Table 3.** Core structures from LPSs of *Agrobacterium* species, LA stands for lipid A backbone and represents the disaccharidic unit: →6)-β-D-GlcN4*P*-(1→6)-α-D-GlcN1*P*

| Species | Structure | Ref. |
|---|---|---|
| *A. larrymoorey* | β-D-Qui3NAcyl-(1→2)-α-L-Rha-(1→4)-α-D-GalAN-(1→6)-α-D-Gal-(1→5)-Kdo-LA<br>4 4<br>β-D-GlcAN-(1→3)-β-D-Glc-1 ⌋ Kdo-2 ⌋<br>Acyl: 3-hydroxy-2,3-dimethyl-5-oxopropylamino | 52 |
| *A. tumefaciens* A1, DSM 30150 | α-D-Man-(1→6)-α-D-Man-(1→5)-Kdo-LA<br>4<br>β-D-Gal-(1→8)-Kdo-2 ⌋<br>Terminal Mannose and Galactose are non-stoichiometric substituents | 53 |
| *A. tumefaciens* TT111, DSM 30204 | α-L-Rha–(1→2)–α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-D-Man-(1→5)-Kdo-LA<br>2 4<br>└1–α-L-Rha β-D-Gal-(1→8)-Kdo-2 ⌋<br>4<br>β-D-GlcN-(1→2)-β-D-Gal(1 ⌋<br>Terminal rhamnose and glucosamine are non-stoichiometric substituents | 54 |
| *A. radiobacter* Rv3, DSM 30207 | α-D-Man-(1→2)-α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→5)-Kdo→LA<br>6 4<br>α-D-Man-1 ⌋ β-D-Gal-(1→8)-Kdo-2 ⌋ | 45 |
| *A. rubi*$^{T}$, DSM 6772 | ┌1-α-L-Fuc α-Dha-(2→3)-α-D-GalA-(1→3)-β-D-Glc-1┐<br>4 4<br>α-D-GlcN-(1→4)-β-D-GlcA-(1→4)-α-D-GalA-(1→3)-β-D-Glc-(1→6)-α-D-Glc-(1→5)-Kdo-LA<br>3 3R 4<br>└1-β-D-Gal-(2←1)-α-L-Fuc α-D-GalA-(1→7)-Kdo-2 ⌋<br>3/4R<br>Acetyls and GalA on external Kdo are non-stoichiometric substituents. R = Ac | 45 |

All monosaccharides are pyranoses, except where specified. Kdo residues are α-configured.

**Table 4.** Core structure from LPSs of *Rhizobium etli* CE3 and structural motifs found for other rhizobial LPSs

| Species | Structure | Ref. |
|---|---|---|
| *Rhizobium etli* CE3 | α-D-Man-(1→3)-β-QuiNAc-(1→4)-Kdo-(2 ┐ ┌α-D-GalA-1<br>└1–α-L-Fuc-(3← O-Chain 6 4<br>α-D-Gal-(1-6)-α-D-Man-(1→5)-Kdo-LA<br>4<br>α-D-GalA-(1→4)-Kdo-2┘<br>5<br>α-D-GalA-1┘ | 11 |
| *Sinorhizobium meliloti* 102F51 | β-D-GlcN-(1→4)-Kdo; α-D-GlcA-(1→4)Kdo<br>α-D-GalA-(1→3)-α-D-Glc-(1→5)-Kdo | 55 |
| *Bradyrhizobium japonicum* 61A101c | α-D-Man-(1→4)-α-D-Glc-(1→4)-Kdo<br>α-D-4OMeMan-(1→5)-Kdo | 56 |

LA stands for lipid A backbone and represents the unit: →6)-[α-D-GalA-(1→4)]-β-D-GlcN-(1→6)-α-D-GlcNonate. All monosaccharides are pyranose, and Kdo residues are α-configured.

links the core region to lipid A, that is, on the presence of particular monosaccharides and the abundance of negatively charged functional groups, which play an important role in the stability of the Gram-negative cell wall by furnishing ionic bridges.[1]

Both *Agrobacterium* and *Rhizobium* LPS core regions share, in most cases, the same residue linked to the position O-5 of the first Kdo, namely α-D-Man or α-D-Glc. The core region of LPSs from *A. larrymoorei* represents an exception because the inner Kdo carries an α-D-Gal unit. The presence of negatively charged functional groups seems to be limited only to the core moiety because no acidic residues have been identified in OPS, as it also holds true in general for the OPS of phytopathogenic bacteria. From this point of view, it is interesting to note that all *Agrobacterium* LPS core regions, except that of *A. rubi*, contain only two Kdo units as acidic monosaccharides, whereas in the *Rhizobium* core region there are additional GalA residues. The occurrence of another acidic sugar, the 3-deoxy-*lyxo*-2-heptulosaric acid unit (Dha), was described for several *Rhizobiaceae*,[18] but its location in the core structure has so far been defined only for LPS of *A. rubi*$^{T}$.

## 2.3. Lipid A

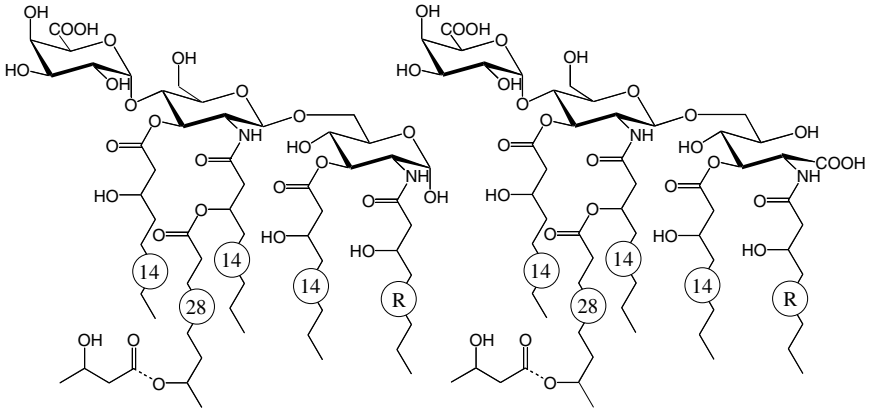
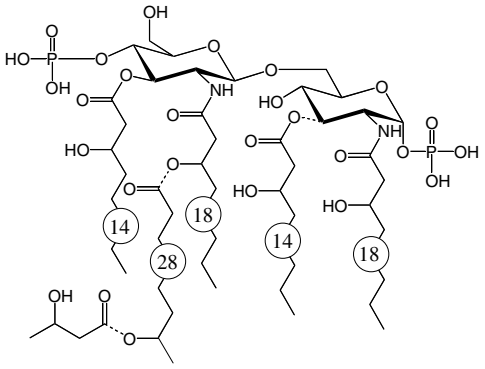
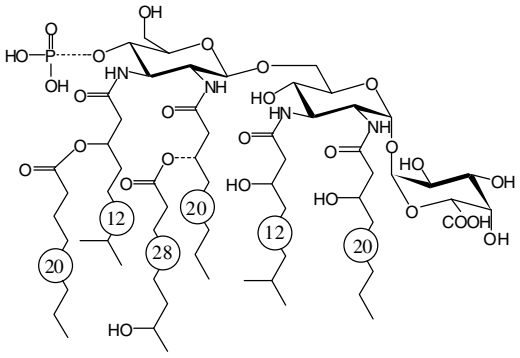
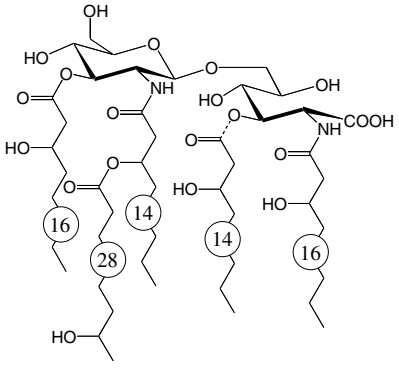
Lipid A is the inner part of LPS, anchoring it in the outer layer of the bacterial outer membrane by means of its long chain acyl moieties. Its architecture is generally considered conserved among individuals belonging to the same family, and from the structural viewpoint, lipid A is a glycolipid typically composed of a 2-amino-2-deoxy-glucose (glucosamine) disaccharide backbone, which is phosphorylated at positions 1 and 4′.[19] Other substituents can either be linked to the phosphates or can replace them. In addition, the disaccharide backbone is acylated by 3-hydroxy fatty acids at position 2, 3, 2′, and 3′ of both proximal and distal glucosamine (GlcN I and GlcN II, respectively). These fatty acids (primary) can be further esterified at their 3-hydroxy group by other fatty acids (secondary). Heterogeneity is mainly related to the nature, number and location of the acyl substituents: a family of hexa-acyl species (lipid A with six fatty acids) differs with regard to the nature of the fatty acids assembled or their distribution on the disaccharidic backbone. Besides the lipidic heterogeneity, the glycidic backbone itself is often not regular, being substituted non-stoichiometrically with phosphate, phosphoethanolammine and/or further sugar residues.

Analysis of the structures reported in Table 5 points out that the only so far described *Agrobacterium* lipid A, that from *A. tumefaciens* C58, shares some structural features with those of other *Rhizobium* species, but key differences are present especially regarding the disaccharide backbone.

The primary fatty acids of *Rhizobiaceae* LPSs are generally saturated, although one unsaturation might be present (in minor amounts not shown in the table), are even numbered with a typical length of 14 or 16 carbon atoms and are always hydroxylated at C-3. Typically, only one secondary fatty acid is present leading to penta-acylated lipid A species, which constitutes one of the most conserved and peculiar motifs, namely octaeicosanoic acid, hydroxylated at C-27 [28:0(27-OH)]. It is worthy to note that this unit is located always in the same position, namely on the amide-linked fatty acid on the distal glucosamine, and it often bears a tertiary acid at its C-27 hydroxyl function, that is, a unit of 3-hydroxybutyric acid. In this regard, *Bradyrhizobium* is even more peculiar because the presence of other very long fatty acids, from 28:0(27-OH) through 32:0(31-OH) have been reported.[20]

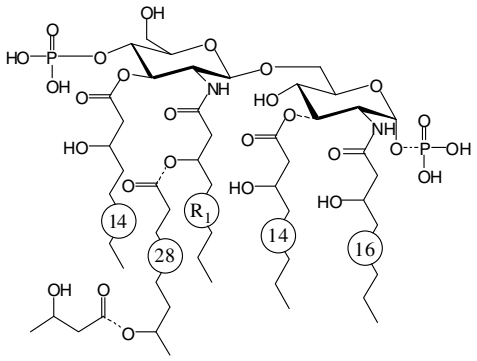
The lipid A of *Mesorhizobium huakuii*[21] represents an exception since this bacterium produces hexa-acylated species carrying an additional non-hydroxylated secondary fatty acid on the non-reducing sugar moiety (see Table 5 and below).

**Table 5.** Structures of the main lipid A components from LPSs of *Rhizobiaceae*

| Species | Structure | Ref. |
|---|---|---|
| *Rhizobium etli* CE3 |  | 24,25 |
| *Rhizobium leguminosarum* bv. *viciae* 3841 | As *Rhizobium etli* CE3 | 27 |
| *Sinorhizobium* sp. NGR234 |  | 23 |
| *Mesorhizobium huakuii* IFO 12543 |  | 21 |
| *Rhizobium* sp. Sin-1 |  | 28 |

(*continued on next page*)

**Table 5** (*continued*)

| Species | Structure | Ref. |
|---|---|---|
| *Agrobacterium tumefaciens* C58 |  | 22 |

The chain length of fatty acids is indicated by a number within the circle. A dashed line indicates non-stoichiometric substituents. R: the fatty acid can have either 16 or 18 carbon atoms. $R_1$: 18:1.

On the other hand, the disaccharidic backbone, and in particular the nature of the sugar constituents appear to be less conserved in LPSs of the different genera and species. The absence of phosphate units is characteristic for several *Rhizobiaceae*; however, a negative net charge is still present in most of the cases originating from the introduction of other groups. Actually, the classical diphosphorylated glucosamine disaccharide backbone has been described only for *A. tumefaciens* C58[22] and *Sinorhizobium* sp. NGR234.[23] A different design of the charged saccharidic backbone was described for *R. etli* CE3,[24,25] whose biosynthesis has been studied in depth and is analogous to that of other *R. leguminosarum* species.[26] Actually, this bacterium produces two different types of lipid A, in both cases, the phosphate at O-4 of the distal glucosamine is replaced by a residue of galacturonic acid, whereas the proximal glucosamine is either present in the reducing form or is replaced by a unit of 2-amino-2-deoxy-gluconate. The proximal 2-amino-2-deoxy-gluconate motif is also shared by other species as *R. leguminosarum* bv. *viciae* 3841[27] and *Rhizobium* sp. Sin-1.[28]

With regard to the disaccharide backbone, *M. huakuii*[21] is rather peculiar having two units of 2,3-diamino-2,3-dideoxy-glucose (DAG) linked β-(1→6). This lipid A lacks the phosphate group at the distal sugar, but the net negative charged is maintained at this position by means of a galacturonic acid residue that is attached by a trehalose type linkage.

## 3. Biological relevance of LPS structures

### 3.1. OPS and core regions

The similarities found for OPS (or core) structures among the species of these two genera apparently do not imply any particular structure/activity relationship since the inclusion of both *Agrobacterium* and *Rhizobium* by the host is different.

In the case of *Agrobacterium*, the first requirement for the disease development is the adsorption of this bacterium on the lower part of the plant, as demonstrated by several experiments in vivo (on bean plants) or in vitro (slices of carrots or potatoes). In both cases, the adhesion process[7] is modulated by the components of the outer membrane of the bacterium, the LPSs, and a correlation among the bacterial effectiveness in the pathogenesis process[29] and the general hydrophobic character of the OPS or of the core region is obvious, as shown in Table 6. These early experiments described the ability of different strains of *Agrobacterium* to induce tumor formations of two plant systems, that is, pinto bean leafs and carrot slices. Typically, a bacterial suspension is

**Table 6.** Correlation among infectiveness data of *Agrobacterium* species and the main constituents of the OPS or core regions

| Species | Pinto bean leaf | Carrot discs | Main sugar constituents |
|---|---|---|---|
| *A. tumefaciens* B6 | 4+ | 4+ | Fuc, Ara |
| *A. tumefaciens* TT111 | 4+ | 4+ | Rha |
| *A. tumefaciens* C58 | 4+ | 3+ | 4OAc6dTal |
| *A. tumefaciens* TT9 | 3+ | 2+ | Rha, Fuc, Fuc4NR |
| *A. tumefaciens* A1 | 2+ | 2+ | Man |
| *A. rubi*$^T$ | 4+ | 2+ | Fuc and acetylated Gal |

Biological assays were performed both on pinto bean leaves and carrot discs, the infectivity ability of each strain was dosed with respect to *A. tumefaciens* B6 and expressed as percentage. *A. tumefaciens* culture dilution (bacterial count/mL/(leaf or carrot tissue)) was chosen to induce between 10 and 50 tumors/bean leaf (or an appreciable amount of tumor-tissue/carrot-disc). The same culture dilutions were used for the other strains tested and according to the results observed, this rating scale was constructed: 4+ implied a range of 80–100% effectiveness of the bacterium compared to *A. tumefaciens* B6 (almost same number of tumors or same amount of tumor tissue on carrot discs), 3+: 20–79%, 2+: 5–19%, +: 0.1–4%.

placed on the plant target and after incubation, the area is rinsed with water and successively the number of tumors is determined and the bacterium infectivity rated with respect to *A. tumenfaciens* B6 is considered as reference strain. Different studies have addressed another important issue, namely the role of the LPSs in this process, utilizing a competition assay based on the pre-treatment of the plant surface with a LPSs solution prior to bacterial inoculation. Actually it was possible to protect the plant by saturating the cell wall receptors with LPSs. When the same area was successively treated with a bacterial suspension, the adsorption of the pathogen and the resulting disease was strongly reduced.[8]

It is worthy to note the difference between the two strains, *A. tumefaciens* TT111 and A1. A classification based on biochemical, physiological and nutritional characters placed these strains in the same group,[30] but differently from the representative strain TT111, A1 possesses very low oncogenic properties, probably due to the absence of hydrophobic sugars in the bacterial membrane.

Currently, no information is available on a biosynthetic modification of the antigenic moieties induced during the host–guest interaction, as was proven for *Rhizobium*.

As far as *Rhizobium* is concerned, it is not possible to generalize the role of the OPS in the symbiotic process, since several experiments showed that this role is most often considered depending on the species.[31] In most cases reported, mutant bacteria with an impaired OPS biosynthesis were effective in the host-plant recognition process but failed at the stage of nodule development, leading to incomplete nodules or to nitrogen non-fixing pseudonodules. These experiments suggested that the OPS is required for the interaction and the final differentiation of *Rhizobia* in bacteroids inside the symbiosome, a vesicle inside the plant cell, where a key feature is the interaction of the bacteria with the lining membrane.

Similar results were obtained studying the *lpsB* mutant of *Sinorhizobium meliloti*, whose oligosaccharide core structure was altered as demonstrated from the lack of uronic acids.[32] This mutant produced a different LPS, rich in a previously absent residue, xylose, and it was found capable of colonizing curled root hairs and forming infection threads in a manner similar to the wild type bacterium. However, developmental abnormalities occurred at the stage of the bacterial invasion of the plant nodule cells.

All the above data prove that the correct OPS (and/or core) structure is important for symbiosis but also that its structure can be finely modified during the bacteroids' development as demonstrated by analysing the bacterial growth in conditions mimicking the root nodule environment.[31,33] Carlson and co-workers[33] have recently demonstrated for *R. etli* CE3 the nature of the chemical modifications of the whole LPSs during this adaptation process. The LPSs were altered in both, the lipid A and the OPS, and in the latter the hydrophobicity was increased by means of O-2 methylation of one fucose residue inside one of the five repeating units present in the OPS moiety.

### 3.2. Lipid A

In mammalian innate immunity, the biological relevance of LPSs resides in the lipid A moiety.[34] In the case of plants, it is accepted that the defence response against potential harmful guests is based on their innate immune system, although the receptors dedicated to the recognition of the lipid A moiety are not identified yet. A number of laboratories have been investigating the ability of rhizobial lipid A to activate both, plant and mammalian innate immune systems, to either parallel the responses of the two systems or find an antagonistic compound with potential pharmacological importance.

In the context of the mammalian innate immune system, the experiments of Moore and co-workers were centered on the ability of LPSs from *R. etli* CE3, *Rhizobium* Sin-1 and *R. galeae* to induce TNF-α in human monocytic cells, using *Escherichia coli* as control.[35] Both *R. galegae* and *Rhizobium* Sin-1 were unable to induce TNF-α synthesis even at mg/mL concentrations, whereas 10 ng/mL concentration of *E. coli* LPS was a potent inducer. An intermediate behavior was observed for *R. etli* CE3, which was active in a concentration range of 100 ng/mL. In addition, it was estimated that the LPS mixture from *Rhizobium* Sin-1 could interact with Mono Mac6 cells with an affinity comparable to that described for *E. coli*, resulting in a good antagonistic activity preventing the binding of the bacteria to Chinese Hamster Ovarian (CHO) cells.

It is worthy to note that similar experiments with *R. galegae* succeeded in patenting the LPS from this organism as adjuvant in vaccine formulation, due to the ability of this molecule to trigger the adaptative immune response without the negative drawback of septic shock, usually experienced with enterobacterial LPS.[36]

Considering the impact of these molecules in the plant system, the peculiar acylation pattern of *Rhizobium* lipid A (long chain fatty acid, low acylation pattern, low phosphorylation) is deemed as a strategy of the bacterium to escape or attenuate the plant response and to fruitfully establish the symbiosis.[37] The structural integrity of this molecular pattern is of importance for the establishment of an accurate symbiosis. In this regard, *S. meliloti* carrying a mutation in the *bacA* gene was studied,[38] the gene encoding for a protein involved in the transport of 28:0(27-OH).[39] This mutant did not assemble this fatty acid in lipid A but was viable and grew in liquid culture, it was more sensitive to detergents and other envelope-disrupting agents, and it was able to

initiate symbiosis to the stage of nodules formation, but it was unable to promote nitrogen fixation. The *bacA* mutant cells lysed soon after endocytosis and did not exhibit morphological changes that were characteristic of bacteroids, such as cell elongation.

Similar results were reported for *R. leguminosarum* bv. *viciae*,[40] mutated in the *acpXL* gene which encodes the acyl carrier protein for 28:0(27-OH). Likewise to *S. meliloti*, this mutant was sensitive to acidic conditions and to increased salt concentration, it grew slowly, it was delayed in nodulation and produced a reduced number of nodules at early postinoculation timepoint; however, it caught up with the parent strain at later timepoints. The nodules were nitrogen-fixing, but they presented a delayed onset of nitrogenase production whose level never reached that of the parent strain. In addition, the mutant bacteroids displayed an irregular shape when compared to the Y-shaped parent strain, and they were unable to multiply synchronously with the symbiosome membrane. All these effects suggested that the mutant was defective in bacteroid maturation, although the mutation was not critical for the outcome of the symbiosis. Later experiments[41] proved that these mutant bacteroids were not completely defective in the nitrogen fixing process, due to their ability to partially restore the addition of 28:0(27-OH) to the lipid A moiety in the nodule environment, by virtue of an alternate mechanism related to another *acp* gene.

Interestingly, a *S. meliloti* strain mutated in the same *acpXL* gene was constructed and studied.[42] The LPS fraction did not contain 28:0(27-OH) and its carbohydrate chemical composition was similar to that of the parent strain, although exclusively LOS species appeared in the SDS-PAGE profile. This apparent contradiction was explained on the basis of different aggregation properties of LPS induced from the lack of the long acyl chain. The *S. meliloti acpXL* mutant displayed sensitivity to deoxycholate, and delayed nodulation of *Medicago sativa*, but nodules elicited by this mutant on the roots of *M. sativa* and *Medicago truncatula* had a normal morphology and fixed nitrogen. Thus, the authors concluded that 28:0(27-OH) of lipid A was not crucial for establishing an effective symbiosis with the host plants, although possible modifications of this moiety during the bacteroid maturation were not investigated.

Finally, an additional important feature considered in a recent paper by Carlson and co-workers,[33] regards the lipid A modification occurring during the bacteroids differentiation of *R. etli* CE3. This bacterium produced a family of lipid A molecules, with a prevalence of penta-acylated forms, but during the differentiation in the nitrogen fixing nodule, the newly synthesized molecules were tetra-acylated and differed for the lack of one unit of 14:0(3-OH), whose position in the disaccharide backbone is still not identified.

## 4. Conclusions

Bacteria belonging to the *Rhizobiaceae* family exhibit a different range of biological activities in the plant kingdom. They can exert a pathogenic effect, as for the *Agrobacterium* genus, or a beneficial symbiotic effect, as for the *Rhizobium* genus.

The interaction of these bacteria with plants is influenced by many factors. In the case of *Rhizobium*, several experiments have evidenced two key points: first, the integrity of the three domains of the LPSs is fundamental for a successful conclusion of the whole symbiotic process,[31,38] and, second, the LPSs architecture is not static, but is finely tuned in response to the nodule formation, introducing subtle but key chemical modifications.[33]

For *Agrobacterium* species, most of the studies aimed to clarify the LPSs role in the pathogenesis mechanism were performed when the structure of these molecules was not known. The importance of the membrane constituents was clearly proved by experiments based on the principle of competition among these molecule and the whole cell.[8]

Thus, bacteria belonging to *Rhizobiaceae* are able to engage apparently complicate interactions with their hosts, and the comprehension at molecular level of the events involved will be greatly supported by the determination of the chemical structure of the main bacterial membrane constituents, the LPSs.

## Note added in proof

Two new *O*-polysaccharides structures were published in 2008, Carb. Res., Vol. 343, 477–482; Biomacromolecules, ASAP Article; DOI:10.1021/bm701011d.

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