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CARBOHYDRATE RESEARCH

Perspective

# Carbohydrate determinants of *Rhizobium*–legume symbioses

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**Abstract**

*Rhizobium* is a genus of symbiotic nitrogen-fixing soil bacteria that induces the formation of root nodules on leguminous plants and, as such, has been the subject of considerable research attention. Much of this work was initiated in response to the question 'how does recognition occur between free living rhizobial bacteria in the soil and potential host legumes?' The answer to this question has been shown to involve both cell-surface carbohydrates on the external face of the bacteria and secreted extracellular signal oligosaccharides. This review will focus on the structure, function, and biosynthesis of two of these components—the host-specific nodule-promoting signals known as Nod(ulation) factors and the rhizobial lipopolysaccharides. 

*Keywords:* Rhizobium; Nod-factors; Lipopolysaccharide; Lipochitooligosaccharide; Symbiosis

## 1. *Rhizobium* nodulation factors

Symbiotic bacteria of the genus *Rhizobium* secrete small oligosaccharide signal compounds called Nod factors that induce the formation of root nodules on leguminous plants [1]. Within these nodules the rhizobia are able to reduce atmospheric nitrogen to ammonia, thus providing the host plant with all of its essential nitrogen requirement. The infectious process often occurs via the plant root hairs, the earliest symptom usually being a deformation or curling of the developing root hairs [2]. This process, and the ensuing nodule development, are host specific, in that a particular *Rhizobium* species will only nodulate a small, defined range of plants. Bacterial mutation analysis has shown that nodule development is controlled by the rhizobial *nod*, *nol* and *noe* (nodulation) genes [3], and that many of these are involved in the production of secreted rhizobial signals called Nod factors [3,4]. The Nod factor alone, either the isolated natural products or synthetic Nod factors, are sufficient to initiate root-hair deformations (at $10^{-12}$ M) and to trigger nodule development (at $10^{-7}$ M), but only on specific host legumes [5–7]. Furthermore, this dramatic biological activity of the Nod factors at least partially defines the rhizobial host range because Nod factors from a particular rhizobial strain are only active on the host legume species of that strain.

Nod-factor biosynthesis and secretion, as determined by the rhizobial nodulation genes,

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are actuated in response to flavanoid signals that are secreted by the host plant [8]. The nodulation genes are often categorized as regulatory (*nodD*), 'common' (*nodABC*) or host-specific (Hsn) [1,3]. The presence of *nodABC* genes in all symbiotically competent rhizobial species suggests that they are involved in the biosynthesis of a common structural feature of all of the Nod factors. Recently, NodC protein has been shown to have *N*-acetylglucosamine β-(1→4)-transferase (chitin synthase) activity [9,10]; NodB to have chitin oligosaccharide terminal *N*-deacetylase activity [11]; and NodA to be an *N*-acyltransferase [12,13]. Concomitant with this is the finding that a common structural feature of almost all the Nod factors reported to date consists of a short oligomer of β-(1→4)-linked *N*-acetylglucosamine residues (generally degree of polymerization 4 or 5) that is N-acylated on the distal glucosamine. This 'common core' may be a biosynthetic intermediate of all Nod factors, which is enzymatically modified in different rhizobial strains under the influence of the various host-specificity (Hsn) *nod* genes, so that different *Rhizobium* species produce structurally varied Nod factors (Fig. 1).

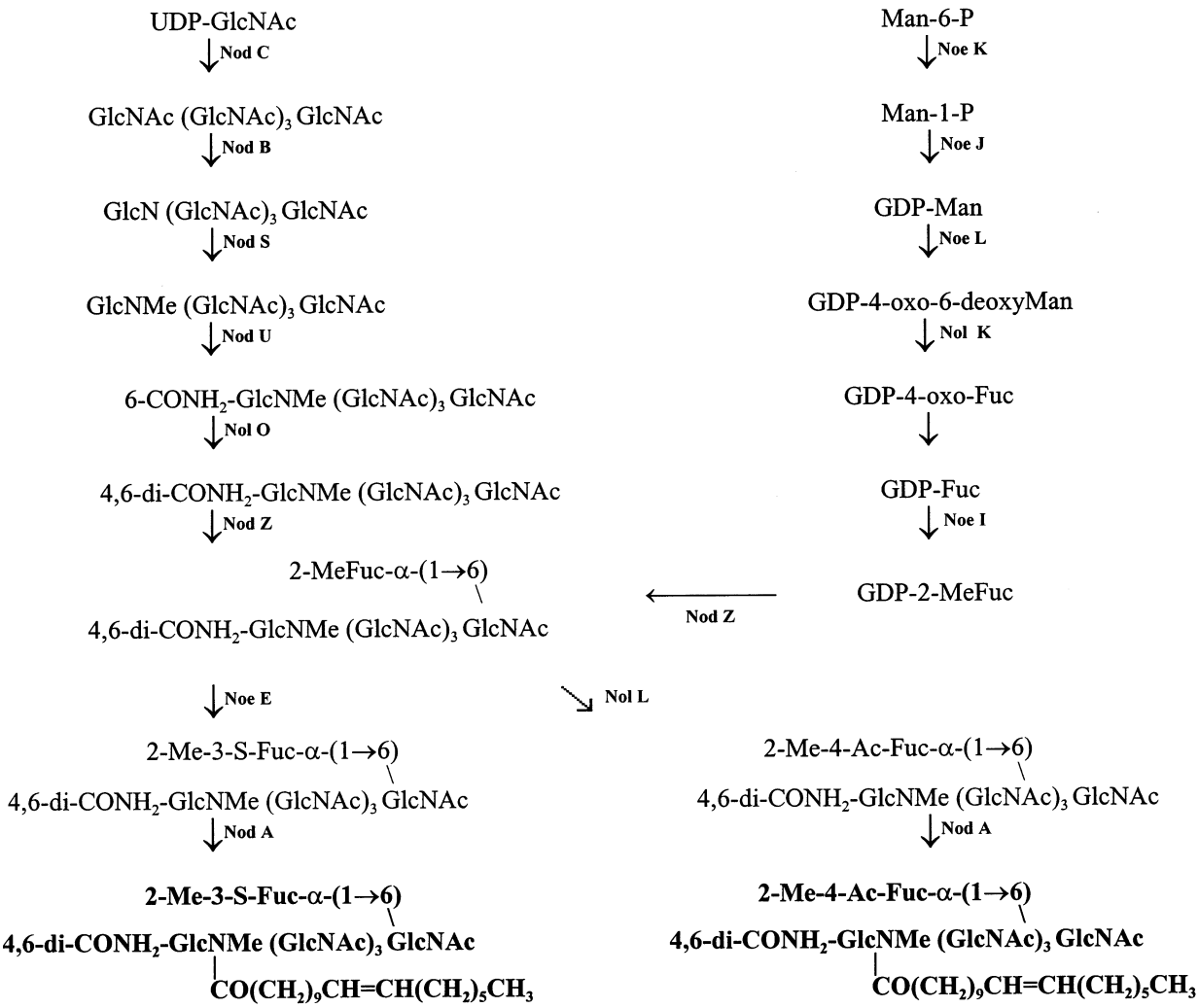


Fig. 1. Biosynthesis of the two major *Rhizobium* NGR234 Nod-factors (structures shown in bold) from UDP-GlcNAc and Man-6-P. Enzymes involved are the following: (1) chitopentaose synthase (Nod C); (2) *N*-deacetylase (Nod B); (3) *N*-methyltransferase (Nod S); (4) 6-*O*-carbamoyltransferase (Nod U); (5) 4-*O*-carbamoyltransferase (Nol O); (6) α-(1→6)-L-fucosyltransferase (Nod Z); (7) phosphomannomutase (Noe K); (8) GDP-Man pyrophosphorylase (Noe J); (9) GDP-Man 4,6-dehydratase (Noe L); (10) GDP-4-oxo-6-deoxy-D-Man 3,5-epimerase/4-reductase (Nol K); (11) 2-*O*-methyltransferase (Noe I); and (12) *N*-acyltransferase (Nod A). Other proteins may be involved in the biosynthesis of the Nod-factor lipid moiety (e.g., Nod E and F) or in secretion (Nod I and J). The pathway is deduced from Refs. [17,18,37,39,40,43].

## 2. Implications of Nod-factor structure on biological activity

To date, over 60 different Nod-factor structures have been identified from 17 different rhizobial strains [14–30]. The so-called 'host-specificity modifications' are confined to the terminal glucosamines of the N-acylated chitomer core, and may include 6-*O*-sulfo, 6-*O*-acetyl, substituted 6-*O*-fucosyl, or 3-*O*-arabinosyl residues linked to the reducing glucosamine, and/or 6-*O*-acetyl, *N*-methyl, and *O*-carbamoyl groups on the nonreducing terminus. Some of these structural modifications to the Nod-factor core have been shown to determine the host range of its biological activity so that structurally varied Nod factors exert their biological effects on different legume species [15,17,19,20,23].

*Substituents to the 6-position of the reducing* N-*acetylglucosamine*.—Eight different 6-*O*-linked substituents can occur on the reducing sugar: (i) sulfate (*R. meliloti*, *R. tropici*, *S. teranga*); (ii) acetate (*R. leguminosarum* biovars. *viciae* and *trifolii*); (iii) L-fucose (*R. fredii* USDA257 and HH103, *A. caulinodans*); (iv) 4-*O*-acetylfucose (*R. loti*, *R. etli*) (v) 2-*O*-methylfucose (*B. japonicum*, *R. fredii* USDA257 and HH103), (vi) 4-*O*-acetyl-2-*O*-methylfucose (NGR234); (vii) 2-*O*-methyl-3-*O*-sulfo-fucose (NGR234); and (viii) the unsubstituted 6-hydroxy group (*R. leguminosarum* bv. *viciae*, *R. tropici*). In addition, *A. caulinodans* Nod factors can have a 3-*O*-arabinosyl unit attached to the reducing residue. Evidence suggests that the 6-*O*-sulfo group is the determinant for recognition by alfalfa, one of the host plants of *R. meliloti*, whereas unsubstituted Nod factors are biologically active on vetch [14,31]. Sulfated Nod factors elicit nodules on alfalfa, whereas nonsulfated Nod factors do not [31]. Corresponding enzyme activity has been demonstrated for a 6-*O*-sulfotransferase from *R. meliloti*, which is encoded by the *nodH* gene [32]. Moreover, *R. meliloti nodPQ* genes specify for the biosynthesis of 3′-phosphoadenosine-5′-phosphosulfate (PAPS), the in vivo substrate for the NodH transferase [33]. *R. meliloti nodH*$^-$ mutants lose the ability to nodulate alfalfa, but gain the ability to nodulate vetch [31,34]. Conversely, transformation of *R. leguminosarum* bv. *viciae* with *nodH* extends its host range to include alfalfa [34]. Furthermore, sulfated Nod factors from *R. meliloti*, but not nonsulfated factors, bind specifically and reversibly to a membrane fraction from alfalfa root hairs [35].

Similar reasoning suggests that the *nodZ* gene from *B. japonicum* USDA110 may encode for a 6-*O*-fucosyltransferase, and that 6-*O*-fucosylated Nod factors are required for nodulation of the forage legume siratro (*Macroptilium atropurpureum*). *B. japonicum* mutants that lack the *nodZ* gene produce nonfucosylated Nod factors and lose the ability to nodulate siratro [36].

Other 6-*O*-linked substituents to the reducing *N*-acetylglucosamine (GlcNAc-1) are required to overcome nodulation resistance. Afghanistan pea cultivar, for example, carries a recessive allele that blocks efficient nodulation by most strains of *R. leguminosarum* bv. *viciae* [21]. However, one strain (designated *R. leguminosarum* TOM) that can nodulate Afghanistan pea cv. has an additional host-specificity gene (*nodX*). Nodulation factors produced by the TOM strain are identical to those of other *R. leguminosarum* bv. *viciae*. strains, except that one is 6-*O*-acetylated on the reducing GlcNAc [21]. Significantly, only penta-*N*-acetylglucosamine Nod factors are acetylated by NodX, and there is no acetylation of tetrameric Nod factors. Thus, a relatively minor structural modification at the 6-*O*-position of GlcNAc-1 is sufficient to alter the biological activity between two cultivated varieties of peas.

Homology of the recently sequenced symbiotic plasmid of *Rhizobium* NGR234 has implicated several other nodulation genes in Nod-factor biosynthesis [37] (Fig. 1). Thus, *noeK*, *noeJ*, *noeL*, and *nolK* determine a biosynthetic pathway from mannose 6-phosphate via GDP-mannose to GDP-fucose [38], the assumed sugar nucleotide donor of fucosylated Nod factors, and *noeI* may be the fucosyl-specific 2-*O*-methyltransferase [39]. Similarly, the *noeE* and *nolL* genes have homology to sulfotransferases and *O*-acetyltransferases, respectively, and are, therefore, implicated in the 3-*O*-sulfonation and 4-*O*-

acetylation of *Rhizobium* NGR234 Nod factors [37,40]. These latter two genes may be coordinately regulated since NGR234 Nod factors that carry both 3-*O*-sulfo and 4-*O*-acetyl groups are not found [17,18].

*Substituents to the 3-, 4- and 6-positions of the terminal nonreducing 2-amino-2-deoxy*-D-*glucose*.—Modifications to the common Nod factor can also occur at the nonreducing terminus involving either differences in the *N*-acyl chain (as discussed below) or *O*-linked substituents at positions 3-, 4- and/or 6. 6-*O*-Acetylation of this residue has been reported in Nod factors from *R. leguminosarum*, *R. meliloti* and *Bradyrhizobium* species, but only for *R. leguminosarum* bv. *viciae* has it been implicated in host specificity [14]. The addition of the *O*-acetyl group appears to be the function of the *nodL* gene, and NodL protein is able to utilize acetylCoA to 6-*O*-acetylate chitin fragments in vitro [41]. NodL-deficient mutants produce nonacetylated Nod factors but, interestingly, these compounds are at 5–10-fold lower concentration than those produced by wild type strains [42].

A less common substituent of sugars, *O*-carbamoyl ($NH_2$–CO–O–), has been identified on Nod-factors from a number of rhizobial species. 6-*O*-Carbamoylation occurs on the nonreducing terminus of Nod factors from NGR234 and *R. loti* species and may also occur at O-3 or O-4 of Nod factors from *A. caulinodans*, NGR234, *B. elkanii*, *R. loti*, *S. teranga* and *R. etli*. Mixtures of di-carbamoylated, mono-carbamoylated and non-carbamoylated Nod factors are produced by *Rhizobium* sp. NGR234 [17,18]. The presence of these carbamoyl groups has been linked to the *nodU* and *nolO* genes [43,39] that respectively determine 6-*O*- and 3-*O*-carbamoylation. If NolO is a specific 3-*O*-carbamoyltransferase [39], then 4-*O*-carbamoylation may arise by a nonenzymatic 3,4 ring migration.

*Substituents to the 2*-N-*position of the terminal nonreducing 2-amino-2-deoxy*-D-*glucose*.—All of the Nod factors identified so far are N-acylated on the distal nonreducing sugar, so that the *N*-acyl substituent may be considered to be part of the 'common core' Nod factor. Non-acylated chitin oligosaccharides (DP 2–6) and a variety of other compounds have neither root-hair deformation (Had) nor nodule-inducing (Noi) biological activities [44]. The 2-*N*-acyl substituent is clearly required for biological activity [14–30]. However, different acyl chains have been identified on Nod-factors from different rhizobial species. Legumes that form so-called indeterminate nodules (such as alfalfa, vetch and leucaena) require Nod-factors with specialized lipid moieties that contain either conjugated double bonds [14,19,30,44] or the presence of the *N*-methyl group [45]. Biosynthesis of conjugated *N*-acyl groups is specified by *nodE* and *nodF* genes which encode for fatty acid synthase and acyl carrier protein, respectively [44], whereas *N*-methyltransferase activity is linked to the presence of the *nodS* gene [45]. Rhizobial mutants that lack the biosynthetic capabilities to produce these structural features lose the ability to nodulate indeterminate hosts [14,45]. By contrast, determinate hosts, such as bean or siratro, are insensitive to the nature of the Nod-factor *N*-acyl group, and simply require N-acylation with prevalent cellular fatty acids such as vaccenate or palmitate.

*Rhizobium leguminosarum* bvs. *viciae* and *trifolii* are a case in point. Nod factors from the *viciae* bv. are characterized by both 6-*O*-acetylation of the nonreducing GlcNAc residue, and by a conjugated polyunsaturated *N*-acyl group, $C_{18:4}\Delta^{E2,4,6,Z11}$. Both of these structural features are required for nodule induction on the indeterminate host plant, vetch, whereas either alone is sufficient to induce root-hair deformations [14]. *R. leguminosarum* bv. *viciae nodEF*-deleted mutants produce *N*-vaccenoyl ($C_{18:1}\Delta^{Z11}$) Nod factors that lack the $\Delta^{E2,E4,E6}$ conjugation system [14], whereas independent deletion of the *nodL* gene results in Nod-factors that lack the 6-*O*-acetyl substituent [41]. The unusual $C_{18:4}$ Nod-factor fatty acid is also associated with certain phospholipids in *R. leguminosarum* bv. *viciae* [46], and the NodA *N*-acyltransferase in this strain is specific for α,β-unsaturated fatty acids [47,48].

There was initially some controversy concerning Nod factors from the *trifolii* bv. [49,50], but it now seems clear that this clover-

specific strain does not produce structures containing the $C_{18:4}\Delta^{E2,E4E6,Z11}$ acyl group [30]. Rather, a complex mixture of Nod factors is made containing a variety of other polyunsaturated *N*-acyl groups, amongst which a *nodE*-dependent $C_{20:4}$ group predominates. Based on these data, it has been proposed that the nodulation host range of *R. leguminosarum* bvs. *viciae* and *trifolii* is determined by the hydrophobicity of the polyunsaturated *N*-acyl moiety of their Nod factors, as determined by the appropriate *nodE* host-specificity gene [51]. Interestingly, a supposedly related strain, *R. etli* CE3 (formerly called *R. leguminosarum* bv. *phaseoli*) produces pentameric *N*-vaccenoyl and *N*-oleoyl Nod factors that totally lack the conjugated *trans*-double bond system [29]. In addition, N-methylation and 4-*O*-carbamoylation of the nonreducing terminus is evident, as well as a 6-*O*-acetylfucosyl group on GlcNAc-1. These Nod factors more closely resemble those from *R. loti* and *Rhizobium* NGR234 than those from *R. leguminosarum* species and, significantly, the bean-specific *R. etli* CE3 strain nodulates determinate-type hosts. Other nodulation factors have been isolated from *R. meliloti* that are N-acylated by $C_{18}$ to $C_{26}$ (ω-1)-hydroxylated fatty acids [52].

In theory, the 22 host-specifying substituents described above could give rise to 484 hypothetical structures—more, if the length of the oligochitomeric backbone is taken into consideration. However, despite this wide structural diversity, there is little evidence to support a 'one Nod factor–one receptive host' hypothesis. Rather, work from the Stacey group suggests a cooperative or synergetic effect, where two or more different structures can induce diverse biological activities on the same host plant species [53]. Nod factors that are glycosidically linked to glycerol have also been isolated from *Bradyrhizobium elkanii* [20]. It may be that these glycerol-linked compounds are the biosynthetic precursor of the Nod factors in this species, because it is known that structural modifications at the GlcNAc-1 anomeric position greatly reduce Nod factor biological activity [54]. Recently, sugar residues other than GlcNAc have been identified as part of the Nod factor's β-(1→4)-linked core, including a mannose reducing sugar [55] and an internal glucose residue [56]. In addition, the major Nod factor from *Mesorhizobium loti* NZP2213 is α-(1→3)-L-fucosylated on an internal GlcNAc residue that is proximal to the terminal *N*-acylglucosamine [27]. The biological consequences of these substituents are as yet unknown but may protect the Nod factor against degradative enzymes of the plant host or in the surrounding environment [57,58].

## 3. *Rhizobium* cell-surface lipopolysaccharides

In common with other Gram-negative bacteria, *Rhizobium* produce a cell-surface lipopolysaccharide (LPS) monolayer through which they interact with the surrounding environment. Because symbiotically associated rhizobia are located intracellularly within host plant cells [2], the rhizobial LPS has received much scrutiny, although only recently have the biosynthesis and a complete structure been thoroughly addressed. Rhizobial LPS is structurally diverse and several species have a naturally rough phenotype that lacks O-antigen altogether. Electrophoretic separation of total rhizobial LPS usually gives two bands: fast-migrating LPS II composed of lipidA-core, and a more heterologous LPS I composed of the LPS II plus attached O-antigen. When present, *Sinorhizobium* LPS I generally migrates as two distinct bands, but the rough LPS II phenotype predominates in laboratory cultured cells [59].

The O-specific chain of *R. etli* CE3, for example, is comprised of GalA, Fuc, (6-deoxy-3-*O*-methyl-D-talose) with a $Me_3$Fuc-Fuc cap (Fig. 2), this being analogous to other rhizobial O-antigens only in the preponderance of methyl and deoxy sugars. A particular *Rhizobium leguminosarum* strain, bv. *trifolii* 4S, has a pentameric repeat unit that consists of α-D-ManNAc-(2→3)-α-L-Rha-(1→3)-α-L-Rha-(1→4)-β-D-GlcNAc-(1→3)-α-L-Rha-(1→ [60]. However, although 4S has part of the tetrasaccharide core that typifies other *R. leguminosarum* strains (exemplified in Fig. 2), it lacks the galactosyl residue to which the O-antigen is usually attached. In the absence of this crucial Gal residue, it has been stated

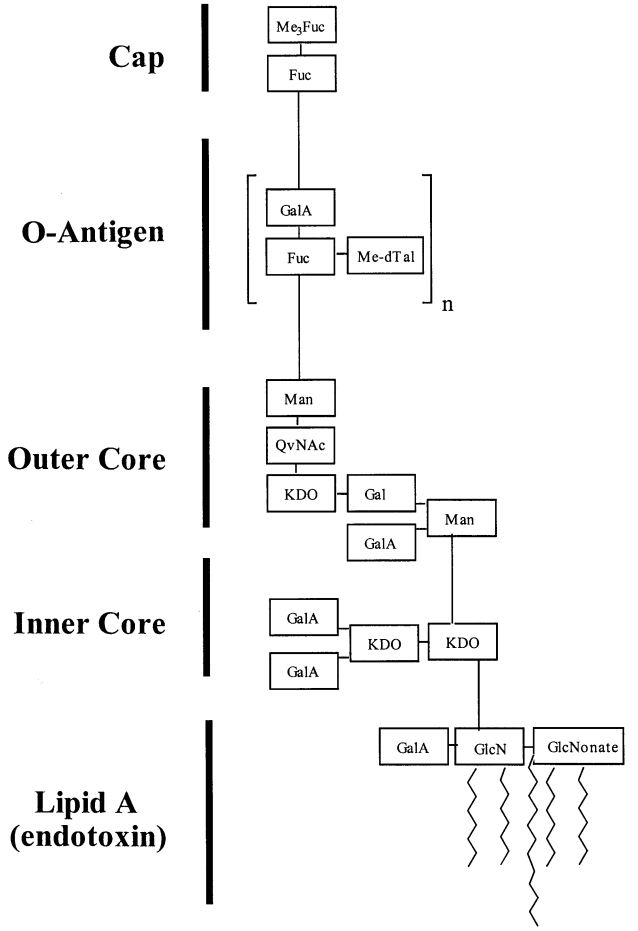


Fig. 2. Schematic structure of the complete lipopolysaccharide from *Rhizobium etli* CE3 [71–73]. Non-standard abbreviations used are for 2,3,4-tri-*O*-methylfucose ($Me_3$Fuc), 6-deoxy-3-*O*-methyl-talose (Me-dTal), *N*-acetylquinovosamine (QvNAc), and 2-aminogluconic acid (GlcNonate). The 2-*N*- and 3-*O*-linked fatty acids present are 3-OH-C14:0, 3-OH-C15:0, 3-OH-C16:0 and 3-OH-C18:0, and the long-chain 27-hydroxyoctacosanoate group is likely 4-*O*-linked to the 2-aminogluconate residue [73] or acyloxyacyl-linked to the 3′-*N*-hydroxy-myristoyl moiety [88].

that the *R. leguminosarum* 4S O-antigen is attached to diacylglycerol rather than to the LPS core [61].

LPS O-antigen from *R. tropici* CIAT899 has been characterized by Gil-Serrano et al. as →4)-β-D-Glc-(1→3)-α-D-2-*O*-Ac-6-dTal-(1→3)-α-L-Fuc-(1→ [62]. A rough mutant of *Azorhizobium caulinodans* (designated ORS571-X15) lacks the dTDP-D-glucose synthase activity necessary for assembly of its O-chain [63]. A *Sinorhizobium* rough mutant, Rm6963, which has low-molecular-weight LPS-I and LPS-II, still induces effective nodules on alfalfa, but seems to provoke a defense response on a second host, *Medicago truncatula* [64]. A similar host-selective effect has been observed previously with LPS mutants of the broad host-range strain *Rhizobium* GRH2 [65]. In this respect, it is interesting to recall earlier work by Schindler and co-workers which showed that purified O-antigen from *Bradyrhizobium japonicum* 110, but not other *Rhizobium* LPSs, inhibits the intercellular transport of hydrophilic, low-molecular-weight substances across the cell wall/membrane interface of neighboring soybean root cells [66]. Transport rate was reduced to 50% by 10 ng/mL LPS, and almost completely at 1 μg/mL. Acetylated fucose and fucosamine are the major glycosyl components of *B. japanicum* 110 O-antigen, together with minor components of xylose and arabinose [67,68].

Partial structures of the LPS core have been established for several *Rhizobium* species [62,69]. The best characterized, *R. etli* CE3 and *R. leguminosarum* bvs., consist of an α-Gal-(1→6)-[α-GalA-(1→4)]-α-Man-(1→5)-Kdo tetrasaccharide and α-GalA-(1→4)-[α-GalA-(1→5)]-Kdo trisaccharide [70,71]. These have recently been shown to be α-(2→4)-linked via the two Kdo residues to comprise a heptameric inner core (Fig. 2) [72]. The α-Kdo-Kdo disaccharide moiety is conserved as in enteric bacteria like *E. coli*, but the *R. etli* LPS core lacks the usual heptosyl residues. Attached α-(1→6) to the Gal is an outer core composed of Man-(1→3)-QvNAc-(1→4)-Kdo, unusual in having a third Kdo residue here in addition to those attached to the lipidA [72]. The O-antigen adjoins the outer core by a (1→3)-linkage and comprises a trisaccharide repeat unit GlcA-(1→4)-[3-*O*-methyl-6-deoxytalose-(1→3)]-Fuc capped by $Me_3$-Fuc-(1→3)-Fuc (L.S. Forsberg, R.W. Carlson, personal communication).

This complete structure is linked to a highly unusual lipidA moiety that lacks the usual 1- and 4′-phosphate groups and acyloxyacyl-linked lauryl and myristoyl groups but rather has a β-(1→4′)-galacturonate residue and an acyloxyacyl or 4-*O*-linked 27-hydroxyoctacosanoate (27-OH-C28:0) group (Fig. 2) [73]. A novel 92-aa cytosolic protein designated AcpXL is the 27-OH-C28:0 acyl carrier during

lipid A biosynthesis [74]. The significance of the long-chain fatty acid is unknown, but it has been noted that it is twice the length of the more usual α-hydroxy fatty acids and may span the entire outer membrane [75]. An alternative point of view is that, although *R. etli* lipidA lacks the usual acyloxyacyl groups, the folding of 27-OH-C28 back on itself would closely mimic these structures. The lipidA disaccharide backbone of the *R. leguminosarum* strains is also unusual in that the proximal residue is 2-aminogluconic acid [73]. By contrast *Sinorhizobium* sp. have the typical β-GlcN-(1→6)-GlcN of enteric bacteria, whereas *Bradyrhizobium* lipidA may contain GlcN plus 2,3-diamino-2,3-dideoxy-D-Glc (DAG) or DAG alone.

Given the structural diversity between *R. etli* and *E. coli* lipidA, it was initially unexpected that the early biosynthetic pathways that convert UDP-GlcNAc to $Kdo_2$-Lipid $IV_A$ are highly conserved, comprising the six enzymes 3-*O*-acyltransferase, deacetylase, 2-*N*-acyltransferase, disaccharide synthase, 4′-kinase, and Kdo-transferase [76]. However, *R. etli* extracts lack late *O*-acyltransferase activities, and instead contain a unique membrane-bound Kdo-activated 4′-phosphatase [77][1]. An *R. leguminosarum* strain that lacks this activity is still able to induce root nodules but is unable to fix atmospheric nitrogen [77,78]. A somewhat less specific membrane-associated 1-phosphatase has also been identified in *R. etli* membranes that removes the 1-phosphate group from $Kdo_2$-lipid $IV_A$ [79].

In accordance with Carlson's assignment of the *R. etli* CE3 lipidA-core, three novel glycosyltransferases, α-(1→6)-galactosyltransferase (LpcA), α-(2→5)-Kdo transferase (LpcB) and α-(1→5)-mannosyltranserase (LpcC), were identified that incorporate the mannosyl, galactosyl and outer Kdo residues in the expected order (Fig. 2) [80]. Interestingly, *lpcC* mannosyltransferase mutants have rough LPS and are unable to properly infect plant nodules [80]. Enlarged infection threads were observed with some intracellular bacterial release but no evidence of bacteroid formation. Similar results have been reported with other rhizobial LPS mutants [81,82], suggesting a possible role in bacterial differentiation or oxygen regulation.

## 4. Integrative carbohydrate metabolism in *Rhizobium* species

Several of the pathways described above use common biosynthetic precursors, and their integrative metabolism needs to be addressed. The utilization of sugar nucleotides and other precursors in more than one pathway may suggest important points of control. For instance, *Sinorhizobium meliloti* secretes 6-*O*-sulfo Nod factors [19] and also has sulfated LPS [83]. Unusually, this species also synthesizes *O*-sulfo-choline as a substrate for glycine betaine biosynthesis [84], suggesting a highly concerted use of metabolic sulfate donors, adenosine-5-phosphosulfate (APS) and phosphoadenosine-5-phosphosulfate (PAPS) [85]. Related strains, *S. fredii* USDA 257 and HH103, both produce heterologous mixtures of α-(1→6)-L-fucosylated and 2-*O*-methyl-α-(1→6)-L-fucosylated Nod factors [24,28]. In addition, USDA257 produces two acidic capsular polysaccharides, one poly-Man-Kdo and the other poly-2-*O*-methylMan-Kdo [86]. Since GDP-L-Fuc is usually biosynthetically derived from GDP-D-Man by way of GDP-L-Gal, one is left with the intriguing possibility of GDP-2-*O*-methyl-D-Man being a direct precursor of GDP-2-*O*-methyl-L-Fuc (see Fig. 1), suggesting a direct biosynthetic link between the Nod factors and capsular polysaccharides [87]. The utilization and regulation of *S*-adenosylmethionine (AdoMet) in this bacterium is clearly an area of future interest.

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