Lipopolysaccharides in diazotrophic bacteria

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INTRODUCTION

With the exception of water, nitrogen is the most limiting compound for plant growth and production. Despite being found in abundance in the Earth's atmosphere as molecular dinitrogen (N₂), it is unavailable to plants which can only use reduced forms of this element, such as ammonia (NH₃). A very specialized group of prokaryotes, named diazotrophs, are able to carry out the conversion of gaseous N₂ into ammonia in a process known as biological nitrogen fixation (BNF), discovered by Martinus Beijerinck in 1901. The BNF process had a major breakthrough in the early 1970's during the oil crisis, when the price of petroleum rose vertiginously, thus affecting the prices of production and transportation of chemical fertilizers. With the aid of BNF plants can readily assimilate NH₃ to produce important biomolecules such as proteins, nucleic acids, ATP, chlorophyll, among others. Diazotrophic microorganisms include aquatic cyanobacteria and free-living bacteria in soil, but a variety of these prokaryotes form associative relationships with plants, and most interestingly, a few have developed an interdependent symbiosis with their hosts, especially legumes, in which specialized group of nitrogen-fixing bacteria. Knowledge gained in the understanding of the molecular basis for these interactions may lead to improving the yield of economically important crops, as well as diminish the impact of chemical fertilizers on the environment by using nitrogen provided by BNF.

GENERAL STRUCTURE OF LIPOPOLYSACCHARIDES

Most Gram-negative bacteria possess LPS as the major component of the outer membrane. Typically, LPS consist of an oligo- or polysaccharide portion, respectively the core and the O-antigen moiety, anchored in the outer leaflet of the bacterium external membrane by a hydrophobic moiety named lipid-A. The latter is structurally conserved among different classes of bacteria, being formed by two units of 2-amino-2-deoxy-D-glucose (GlcN) linked by a β-(1→6) glycosidic bond and phosphorylated at positions 1 and 4’ (Zähringer et al., 1999; Trent, 2004; Wang and Quinn, 2010). Long-chain acyl groups are found either esterifying free hydroxyl groups or N-linked as amide-type substitutions on C2 of both GlcN units (Trent et al., 2006; Raetz et al., 2007). The oligosaccharide core is usually bound to the lipid-A by a Kdo unit (3-deoxy-D-manno-octulosonic acid) linked at C6 of one of the GlcN units (Raetz and Whitfield, 2002). The core varies in monosaccharide composition, but the presence of Kdo (or its derivative, Ko—D-glycero-D-talo-octulosonic acid) is almost mandatory. Some species contain D-glycero-D-mannoheptose.
Serrato Lipopolysaccharides in diazotrophic bacteria (D,D-Hep) alone, or in combination with the most commonly found L,D-Hep, while others may have a diversity of hexopyranoses and aminosugars (Zähringer et al., 1999; Holst, 2011). Within a genus or family the structure of the inner core tends to be conserved, and the fact that distantly related bacteria share structural features is a reflection of the importance of the core in outer membrane integrity (Raetz and Whitfield, 2002). The outermost part of the LPS, the polysaccharide chain or O-antigen, lies in the interface between the bacterium and its surrounding environment, and is where the most structural heterogeneity is found. The enormous structural diversity of O-antigens lies on monosaccharide composition, glycosidic linkage position, size of repeating unit, and chain length, as well as on non-carbohydrate substitutions that may occur (Lerouge and Vanderleyden, 2001; Raetz and Whitfield, 2002). O-antigen modifications seem to play and important role at several stages of the infection process during plant-microbe interactions, including adherence, bypassing or overcoming host defenses, and establishing and maintaining intercellular communication (Knirel, 2011).

Figure 1 shows a schematic model of the general structure found for LPS in Gram-negative bacteria. The complexity of LPS reflects the difficulties encountered to determine their fine structures. In many cases, only the structure of the predominant polysaccharide backbone is known. LPS extraction from bacterial cultures may also be affected by culture age and growth condition. In the case of plant-associated bacteria, culture conditions may be inadequate in order to observe the LPS present during interaction.

LIPOPOLYSACCHARIDES IN RHIZOBIACEAE

Among all diazotrophic bacteria, those belonging to the family Rhizobiaceae have certainly the greater number of species studied in regards to their LPS. Extensive work has been done on structural characterization, biosynthesis and involvement of LPS during Rhizobia-legume interaction (Carlson et al., 1999, 2010; Price, 1999; Noel and Duelli, 2000; Fraysse et al., 2003; Kesawat et al., 2009). Lipid and monosaccharide composition in LPS found for Rhizobiaceae vary considerably, but the basic architecture for this molecule is conserved (Kannenberg et al., 1998). The LPS produced by Rhizobium etli, strain CE3, and R. leguminosarum have the same basic lipid-A backbone. Instead of the typical GlcN disaccharide, both structures are formed by a trisaccharide containing GlcN, GalA, and GlcNate (gluconate) (1:1:1) (Carlson et al., 1999) (Figure 2A). In this case, the phosphate in position 4’ is replaced by a galacturonic acid unit, and both GlcN and GlcNate are N-acylated at C2 and O-acylated at C3 by β-hydroxyfatty acids of different chain length (Bhat et al., 1994). Most lipid-A structures found in Rhizobiaceae, including R. etli, have very-long-chain fatty acids such as 27-hydroxyoctacosanoic acid (27-OH-C28) (Hollingsworth and Carlson, 1989; Kannenberg et al., 1998) (Figure 2B). The inner core of R. etli CE3 is formed by a complex highly-branched octasaccharide containing Kdo, Gal, GalA, and Man, while the outer core that binds the O-antigen has Fuc, Man, and QuiNAc (N-acetyl-quinovosamine) (Forsberg and Carlson, 1998). Despite the structural variations found in the O-antigen, the presence of deoxy-hexoses, methylethyl hexoses, 6-deoxy-amino-sugars, and N-methyl-6-deoxysugars is common together with the presence of acetyl substituents in the structure (Schnaitman and Klena, 1993). The O-antigen of the LPS described for R. etli CE3 has a trisaccharide repeating unit on its terminal portion formed by GlcAp, Fucp, and 3Me-6dTalp (3-methyl-6-deoxy-talose). A cap unit of 2,3,4-tri-O-methyl-fucose is also found as non-reducing terminal (Bhat and Carlson, 1992; Forsberg and Carlson, 1998; Forsberg et al., 2000).

The nodulation process during symbiosis of rhizobia with legumes seems to be affected by the presence of truncated LPS or by the complete lack of these molecules (Carlson et al., 1995).
Genes related to LPS expression and biosynthesis are modulated during symbiosis, and LPS structures are modified during the transition of free-living cells to nodule bacteroids (Broughton et al., 2006). These changes may be induced by plant extracts, and especially by flavonoids (Duelli and Noel, 1997). Mutants of rhizobia deficient in LPS biosynthesis remain on the infection thread during the early stages of nodulogenesis and are unable to complete cellular differentiation into mature nitrogen-fixing bacteroids (Noel et al., 1986; Campbell et al., 2002; Broughton et al., 2006). Mutants of R. etli that produce truncated LPS structures have promoted the growth of deformed nodules without the ability to fix nitrogen (Noel and Duelli, 2000). It has been proposed that LPS in rhizobia are not involved in the early stages of symbiosis (attachment, root hair curling and infection thread development), but have a central role in maintaining viable differentiated cells once de nodules are formed (Kannenberg et al., 1998; Noel et al., 2000). Furthermore, bacteroids of Rhizobium leguminosarum found inside the nodules of some legumes show drastic alterations in their LPS structures in comparison to the structures found for the non-differentiated cells (Goosen-Deroo et al., 1991; Kannenberg and Brewin, 1994). Bacteroids of R. etli and Sinorhizobium meliloti found in nodules of their respective plant hosts have structural differences in the O-antigen of their LPS structures similar to those found when these bacteria are cultivated in low levels of oxygen and low pH, indicating that changes in LPS structure may be due to physiological conditions to which they are exposed (Tao et al., 1992; Kannenberg et al., 1998; Reuhs et al., 1999). These data indicate that the degree of structural alterations on rhizobial LPS influence the chances of bacteroid survival and guarantees the development of an adequate nitrogen-fixing nodule on the plant host (Carlson et al., 1995).

LIPOPOLYSACCHARIDES IN ASSOCIATIVE AND ENDOPHYTIC DIAZOTROPHS

Other than nodulating rhizobia, diazotrophic bacteria are also found associated with roots and rhizosphere, and even inside plant
tissues. This now well-known class of nitrogen-fixing bacteria, capable of establishing endophytic associations with economically important cereals and forage grasses, such as wheat, rice, sugarcane, and maize, has been investigated in recent years with regards to their LPS structures and function during the infection process. To what concerns the structure of LPS during plant-bacterium interactions, some reports have shown that different portions of these molecules may be involved in different stages of the infection process. In Pseudomonas syringae, the loss or alteration of the O-antigen structure is related to an impaired virulence (Smith et al., 1994). Some works have reported the role of LPS in the adhesion process of Agrobacterium tumefaciens to their host cells (Pueppke, 1984; Matthysse, 1986). Mutants of this bacterium that produce LPS with an altered core structure but that maintain a non-defective O-antigen are still able to attach normally to carrot root cells (Mets et al., 1991), showing that the total structure of the LPS is not necessary to the process. The LPS produced by several strains of Herbaspirillum was analyzed by Serrato and coworkers (Serrato et al., 2010) showing that the LPS produced by H. seropedicae SmR1 was different in monosaccharide and fatty acid composition when compared to other strains. Later, the structure of the lipid-A portion of the LPS isolated from strain SmR1 was determined as having a typical β-(1→6)-linked GlcN disaccharide backbone, both units phosphorylated and decorated with units of 4-deoxy-4-amino-arabinose (4NAra) (Fedonenko et al., 2002). Immunochemical interactions, some reports have shown that different portions of these molecules play during the plant-diazotrophic molecular interaction.

CONCLUSIONS

BNF performed by diazotrophic bacteria has been extensively studied over the past decades, as have the symbiotic and associative processes that allow these microorganisms to invade plant tissues and deliver ammonia together with other growth-promoting substances. Even though the role of glycans and glycoconjugates, such as LPS, have been determined for some species during the infection and colonization process with their plant hosts, there are several gaps in the process that are poorly understood and require more investigation. The recent availability of numerous nitrogen-fixing bacteria genome sequences, allied to the chemical, and structural characterization of LPS, offer the tools to determine the functional aspects that these molecules play during the plant-diazotrophic molecular interaction.

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Rhizobium leguminosarum

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