found for each of these problems to enable the extension of site-specific chemical labeling in vivo. Can you then imag(e)ine the possibilities?

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An Abundance of Nodulation Factors

In this issue of *Chemistry & Biology*, Morón et al. [1] report that *Rhizobium tropici* CIAT899 produces different Nod factors in response to flavonoid induction under differing environmental conditions. This unanticipated environmental dependence has implications for altering or potentially improving the host-bacteria interaction in bean nodulation.

Beans are important diet ingredients for humans in developing countries, such as in Latin America and Africa. However, bean growth is often limited by poor soil conditions, e.g., in tropical regions by acidic conditions, and by bean plants' relatively limited capacity for fixing nitrogen in symbiosis with *Rhizobium* bacteria [2].

Legume plants benefit from symbiosis with rhizobia. Inside the symbiotic root nodules, the bacteria reduce nitrogen to ammonia and supply it to the host. Strain *R. tropici* CIAT899 is interesting for several reasons: it is a broad-host range strain and is capable of forming nodules on several legume hosts [3]; it is acid tolerant and able to grow under moderately acidic conditions [4]; and finally, it is capable of nodulating beans under acidic conditions that reduce or suppress nodulation by other strains [5]. These are important traits with implications for agricultural practices. In the past, this versatility was recognized for its importance in understanding the underlying signaling mechanisms in symbiosis [6].

The fundamentals of the underlying signaling mechanisms are relatively well known. *Rhizobium* bacteria perceive a host-specific aromatic signal molecule, e.g., a flavonoid like apigenin [1], in the exudates of the legume hosts. This release leads to the activation of a positive transcriptional regulator, the NodD protein, which in turn leads to the expression of the rhizobial *nod* genes. Once induced, the products of the *nod* genes synthesize molecules called Nod factors or lipochitin oligosaccharides (LCOs) that act as bacterial signals to the host plant and induce the formation of the nitrogen-fixing root nodules [7]. The effects caused by purified LCOs on legume roots are complex. They include cortical cell division and the formation of nodule primordia, altered root hair tip growth, cytoplasmic streaming and rearrangement of actin filaments, and the induction plant gene transcription [8–10].

The Nod factors produced by various *Rhizobium* bacteria are similar to one another with regard to their general structural features (see Figure 1). Normally, Nod factors are comprised of a backbone oligomer of four to five β-(1$\rightarrow$4)-linked *N*-acetyl-D-glucosamine (GlcNAc) monomers. The GlcNAc residue at the terminal nonreducing end carries an *N*-linked acyl chain. The structure of the acyl chain can vary as well as the backbone oligomer through a variety of additional substitutions at the nonreducing terminal N-acyl-GlcN and the reducing end GlcNAc of the Nod factor, depending on the rhizobial species and its combination of *nod* genes. The structure of the acyl chain and substitutions on the GlcNAc backbone are important for the biological activity of the Nod factor and are major determinants of the rhizobial-legume host specificity and the resulting host-specific nodulation [7–9]. Nod factor recognition and perception is currently an intense area of research [8–12].

Observations leading to the elucidation of Nod factor functions have been, to a large degree, obtained from *Rhizobium* bacteria that have a narrow host range and

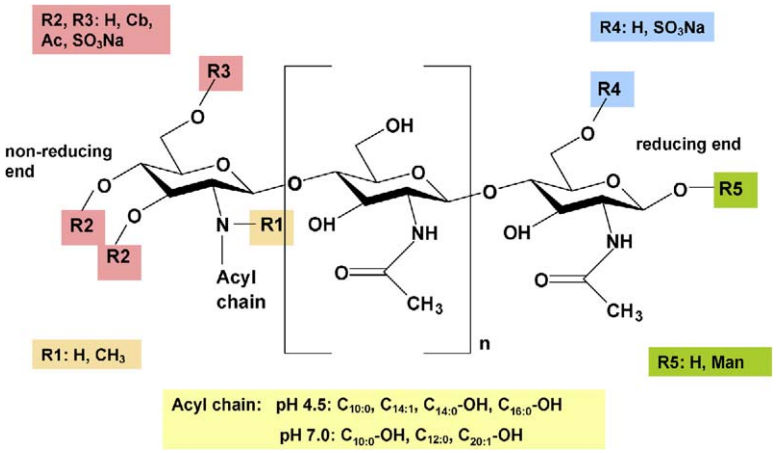

Figure 1. Structural Variability of *Rhizobium tropici* CIAT899 Nod Factors

As with Nod factors of other *Rhizobium* strains, the Nod factor of *R. tropici* CIAT899 is comprised of a *N*-acetyl-D-glucosamine (GlcNAc) backbone with an attached fatty acyl chain at the nonreducing end. A summary of the modifications to the basic structure of this strain is given in the differently colored boxes. Cb, carbamoyl group; Ac, acetyl group; SO_3Na, sulfate group; H, hydrogen; Man, mannose; n, 2 or 3 GlcNAc residues; Acyl chain, fatty acid attached to GlcNAc backbone; acyl chains exclusively observed at pH 4.5 or pH 7.0 are shown in the picture. Acyl chains of the following length were observed at both pH values: $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:0}$-OH, $C_{18:1}$, $C_{18:1}$-OH, $C_{20:0}$, $C_{20:0}$-OH, $C_{20:1}$ ($C_{x:y}$, where x is the number of carbon atoms and y is the number of double bonds of the acyl chain; $C_{x:y}$-OH, acyl chain containing a hydroxy group).

a relatively narrow spectrum of Nod factors. The situation can be quite different if a *Rhizobium* is capable of nodulating different hosts with presumably different Nod factor requirements. In the case of *R. tropici* CIAT899, it was reported that this strain produces 16 different Nod factors [6]. Not surprisingly, this variety of structures has been interpreted as necessary for the nodulation of different legume hosts. For example, while it is thought that an LCO carrying an acyl chain at the nonreducing end of the molecule is sufficient for bean nodulation [2], based on nodulation phenotypes of mutants unable to display certain LCO modifications, it has been concluded that a Nod factor essential for the nodulation of the additional plant host *Leucaena* needs, in addition, a sulfate group [6]. However, the functions of only a fraction of the observed Nod factors have been explained in this way. The biological functions of the larger portion of the various Nod factor structures are as yet unexplained.

The study by Morón et al. [1] utilizes advanced spectroscopic methods to analyze Nod factor production in bacterial cultures and opens a Pandora's Box of additional Nod factor biological complexity. Apigenin-induced *R. tropici* CIAT899 growing under acidic culture conditions produced 52 Nod factors, while at neutral pH the bacteria produced 29 different Nod factors, with only 15 common to both conditions (Figure 1). Among the Nod factors identified under acidic conditions was a hitherto unknown structure that is double sulfated in its backbone [V($C_{18:1}$, NMe,S,S)]. Additional novel *R. tropici* CIAT899 Nod factor structures were produced at neutral pH, such as molecules containing a carbamoyl group or molecules containing mannose as part of the molecular backbone [V($C_{10:0}$-OH,Cb); V($C_{10:0}$-OH, NMe,Cb); IV-Hex($C_{10:0}$-OH,Cb); IV-Hex ($C_{12:0}$,NMe); IV-Hex($C_{18:1}$,NMe)]. Based on the relatively intact nodulation abilities of *R. tropici* CIAT899 under acidic plant growth conditions [1], the authors speculate that this variety of factors might help to minimize adverse effects of the acidic soil conditions on nodule development, or might impact Nod factor stability under acidic conditions. However, experiments confirming these speculations have not yet been performed.

The value of the paper lies in the finding that the production of Nod factors can vary a great deal depending on the environmental conditions the bacteria encounter. The fact that biotic and abiotic factors effect Nod factor production is not new [2], but has, to date, not been investigated systematically. Morón et al. provide the first report showing that acidic conditions increase rhizobial Nod factor production. The paper is largely descriptive, and while it does not address the molecular or regulatory mechanisms underlying the biosynthesis of the large variety of Nod factors or their functional aspects, it does raise many important issues surrounding Nod factor structural complexity.

First, it emphasizes that rhizobial physiology and ecology are underappreciated aspects of Nod factor biology. Second, this paper highlights conditions contributing to Nod factor biosynthesis that have not yet been recognized or studied, such as the influence of pH on Nod factor production. Thus, this work will likely lead to the discovery of more genes involved in Nod factor production as well as to new regulatory mechanisms. Third, these results suggest that the biologically relevant Nod factors for a particular *Rhizobium*-legume host symbiosis may be habitat dependent and more complex than currently thought. This implies that examination of Nod factor function in legume plants should include consideration of soil and root environmental conditions. Finally, the work raises the question of the functional importance of different Nod factors; since chemical synthesis or isolation of specific Nod factor structures for functional studies may only be possible for a small portion of the numerous Nod factors, these new findings emphasize the value of the advanced genomic tools now available that will help to identify additional genes and gene regulation mechanisms involved in environmentally regulated Nod factor biosynthesis. This approach, in turn, will allow for the creation of novel and more subtle Nod factor mutants to examine for their effect on symbiotic function, potentially opening up new avenues in understanding Nod factor functions which may contribute to the long-standing goal of improving bean nodule's poor nitrogen-fixing abilities.

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