RHIZOBIUM SP. BR816 PRODUCES A MIXTURE OF CLASSICAL NOD FACTORS AND NOVEL NOD FACTOR LIKE STRUCTURES WITH A *N*-ACETYL GLUCOSAMINITOL AS THE REDUCING SUGAR

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An efficient symbiosis between rhizobia and leguminous plants involves a multistep reciprocal signal-exchange process. The secreted lipochitooligosaccharides or Nod factors (NFs) are the main determinants of host specificity. With their specific decorations, NFs trigger cortical cell division only in compatible legume plants, leading to nodule formation. The genes required for synthesis and transport of NFs are the nodulation (*nod*) genes. The broad host range strain, *Rhizobium* sp. BR816, isolated from the nodules of the tropical tree *Leucaena leucocephala* nodulates bean (*Phaseolus vulgaris*) and other tropical leguminous plants (Hernández-Lucas et al., 1995). Our research group identified and characterised the different regulatory and structural nodulation genes of BR816. To elucidation its host-specific determinants, we determined the NF structures of this strain.

LCOs were purified from 6-liter apigenin-induced cultures of *Rhizobium* sp. BR816 by using amberlite XAD-4. Adsorbed components were eluted with methanol, dried and further purified on an open C-18 reversed-phase column. After elution with 50% acetonitrile, the components were separated by C-18 reversed-phase HPLC with a gradient of 20% aqueous acetonitrile to 100% acetonitrile in 50 minutes. Fractions of interest were extracted again with butanol. The mass spectra obtained with FAB^{+}-MS and FAB^{-}-MS, together with additional chemical analysis, showed at least three families of Nod factors. Each family consists of members separated by 2 mass units [$(M+H)^{+}$ ions: 1393-1395-1397, 1435-1437-1439, 1477-1479-1481]. All are pentamers with common C18:1 (*m/z* 1393, 1435 and 1477) or C18:0 fatty acids (other *m/z* ions). They are all *N*-methylated and C-6 carbamoylated at the non-reducing end and C-6 sulfated at the reducing end. A second acetyl group can be substituted on the C-3 or C-6 of the *N*-acetylglucosamine coupled to the non-reducing sugar. FAB^{-}-MS carried out before and after hydrogenation of the Nod metabolites showed components still differing by 2 mass units, indicating that this is not due to the presence of unsaturated fatty acids. Monomeric sugars were obtained after peracetylation of the Nod metabolites followed by hydrolysis of the β-(1,4) bridges. GC-MS indicated that the two supplementary mass units may be due to a reducing end sugar without ring formation or a glucosaminitol, which could be acetylated.

BR816 possesses one copy of *nodSU* and *nodH*, encoding proteins involved in methylation, carbamoylation and sulfation of the Nod factor backbone. In BR816, one *nodFE* copy has been characterised and hybridisation studies with heterologous *nodEF* genes suggest the possibility of two more *nodEF* copies (van Rhijn, unpublished). The *nodEF* genes likely determine the nature of the acyl chain linked to the core molecule (Spaink et al., 1991), however, no polyunsaturated fatty acids were detected. A gene encoding an acetyltransferase has not yet been identified. We will now this system to test the functionality of known nodulation genes in BR816. The discovery of a new type of Nod factor molecule opens up the search for new genes in the *Rhizobium* signalling.

References

Hernandez-Lucas I et al (1995) Appl. Environ. Microbiol. 61, 2775-2779.
Spaink H et al (1991) Nature 354, 125-130.