CELL SURFACE GLYCOCONJUGATES OF RHIZOIUM AND SYMSIOSIS

TECHNICAL SUMMARY OF ACTIVITY AND RESULTS
CLOSEOUT OF DE-FG02-89ER14029

During the course of this D.O.E-funded work we studied the roles cell surface carbohydrate components of *Rhizobium* play in determining the outcome of the symbiosis between these microorganisms and plants. Our approach was multidisciplinary and involved the use of structural and synthetic chemistry, biochemistry, genetics and bacterial physiology. A variety of rhizobial cell surface and membrane carbohydrate-containing molecules are believed to function in the establishment of symbioses between rhizobia and legumes. They include extracellular polysaccharides (EPSs), capsular polysaccharides (CPSs), lipopolysaccharides (LPSs), chitolipooligosaccharides (*Nod* Factors), cyclic b-glucans and glycolipids. CPSs or EPSs are widespread and abundant extracellular heteropolysaccharides made up of oligosaccharide repeating units. These polysaccharides either accumulate on the surface of the cell as a capsule (CPSs) or are sloughed off into the cell's surroundings (EPSs). Specific mutations of bacterial genes involved in the various stages of the biosynthesis of these molecules show a high correlation with symbiotic deficiencies in nodulation (1).

LPSs are located exclusively in the outer leaflet of the outer membrane of Gram-negative bacteria. These molecules have three covalently linked regions. They are the membrane embedded lipid A, an oligosacchride core and an antigenic polysaccharide chain (O-antigen). LPSs have been reported to be involved in several stages of symbiosis. These stages include initial infection, bacterial release from the infection threads and bacteroid transformation and maturation (2-8).

Chitolipooligosaccharides are named *Nod* factors because their biosynthsis is controlled by nodulation genes and they are believed to be the primary host range determinants (9) We have demonstrated that these molecules are membrane localized since the quantities recovered from cell pellets are much larger than the quantities in the supernatant (10-11).

DISCLAIMER

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Cyclic b-glucans are unique molecules that are found almost exclusively in bacteria of the *Rhizobiaceae* family. These molecules are oligomers of glucose linked by a -1,2-linkages in rings as large as 20 monosaccharide units or more. They are thought to involved at several stages of plant infection and proposed roles include conferring host specificity and suppression of host defense responses (12).

There are other glycoconjugates that might somehow be involved in the symbiosis between *Rhizobium or Bradyrhizobium* and legumes. We have found that one mannose and glucose-containing diglycosyl diacylglycerol accumulates in the membrane of *Rhizobium leguminosarum* bv. *viciae* ANU843 when the bacteria are grown in the presence of flavonoids (13). Subnanomolar concentrations of this glycolipid could also elicit various symbiosis-related morphological responses such as root hair deformation and cortical cell division on the host plant.

This diversity of bacterial cell surface carbohydrates that are involved in symbiosis complicates studies aimed at defining the roles of each individual components. These studies become even more complicated because of the large degree of coupling or overlap of biosynthetic events leading to the synthesis of the various molecules. For instance, there are common structural (and genetic) links between chitolipooligosaccharides, LPSs and general membrane lipid chemistry (9). Rhizibia with sulfated chitolipooligosaccharides also have sulfated LPSs. Their common origins of sulfation have been demonstrated by using mutants which are impaired in sulfating chitolipooligosaccharides. In such cases, there is a corresponding lack of sulfation of the LPS. The structural diversity of the fatty acids observed in chitolipooligosaccharides is also observed in other membrane phospholipids and glycolipids. The LPS of *R. leguminosarum* bv. *trifolii* 4S does not have the components typically found in other rhizobial lipopolysaccharides. However, we have demonstrated that in this strain, the "missing" typical O-antigen is transferred to an alternative glycolipid (14). Similar links exist between EPS and LPS biosynthesis (15-17) Rhizobial *exo* mutants with an altered EPS often also have a different LPS. There is some evidence that LPS and EPS (or some other capsular antigen) might have the same function in the plant-bacteria interaction in some symbiotic systems (18-21).

Understanding carbohydrate metabolism is, of course, central to understanding the connections between biosynthetic events leading to the various polysaccharides, glycolipids and glycoconjugates which all appear to be so central to the symbiotic process. To this end, we have been engaged in studies to characterize the detailed structures of the various rhizobial glycoconjugates and other saccharides and to delineate the connections between their biosynthesis. These studies have turned up some very important and interesting results. We have discovered that one of the glycolipids synthesized by *Rhizobium* is sulfoquinovosyl diacylglycerol, a molecule once thought to be restricted to plants and photosynthetic bacteria (22). We have found that the predominant lipid in the membranes of *Bradyrhizobium* bacteroids is digalactosyl diacylglycerol, another typical plant lipid not found before in bacteria (23). Another finding was that the predominant lipid in free-living bacteroid-like states of this organism is also a diglycosyl diacylglycerol along with a ribosylated version (24). We now know that these glycolipids are localized on the outer membrane of the cell with the exception of the chitolipooligosaccharides which are localized in the inner membrane (25). We also now know that the culturing *Bradyrhizobium japonicum* under low oxygen conditions triggers the biosynthesis of phosphatidyl inositol, another lipid common in plants and not usually found in bacteria (26). The predominant phospholipid in the membranes of vegetative cells of *Rhizobium* is phosphatidyl choline, another lipid that is common in plants and other eucaryotic systems but quite rare in bacteria. In addition to these findings, we have also demonstrated that considerable carbohydrate antigen switching occurs on the surface of *Rhizobium* as a consequence of lowering pH in some cases resulting in the synthesis of a new lipopolysaccharide component with (27).

In earlier work, we used several immunochemical methods to better define the identity and distribution of carbohydrate species on the bacterial cell surface. One of our major accomplishments was to develop antibodies to the two major polysaccharide antigens which are associated with the cell surface of *Rhizobium*. These are the capsule (CPS), and lipopolysaccharide (LPS). The lipopolysaccharide was separated into three major structural components: a core trisaccharide, a tetrasaccharide and an O-antigen. These components were then converted to allyl glycosides and polymerized into high molecular weight polymers containing a very high density of the haptens. The polymers proved to be excellent immunogens

and antibodies with very high titers against the original haptens were obtained. These antibodies were then used to study the expression of the antigens on the bacterial cell surface at various stages of the symbiosis both *ex planta* and *in planta* using both fluorescence and electron microscopy. This work formed the basis of the Ph.D. degree of Maria Beconi (1995). This work demonstrated that the capsule of the bacteria is shed in the early stages of the infection process and that the LPS epitopes are then exposed but some are polarly located on the cell surface. As part of this project, we complemented this immunological study with a chemical one in which we examined the chemical basis for the observed changes using bacteroids induced by altering pH, oxygen levels, growth conditions and carbon and nitrogen source and levels. Results from this research have been presented at two international conferences.

Another of our major goals was to develop methods for the structural analysis of carbohydrates which may be present in very low concentrations. This was accomplished by the development of the 2,4-dinitrophenyloctylamino derivatives (among others) for the sensitive HPLC detection and mass spectrometric characterization of oligosaccharides. We have since expanded this methodology to include a wide variety of heterooligosaccharides. This work forms the bulk (along with other *Rhizobium* - related work) of the Ph.D. thesis of Yuanda Zhang.

Our ability to determine the structures of complex lipids and glycolipids (especially in mixtures) by NMR spectroscopy has received a tremendous boost by the introduction of an NMR solvent which gives very high resolution spectra across the full spectrum of lipid classes. This is a significant and necessary development.

One of our major goals was to completely characterize the cell surface carbohydrate chemistry of a few strains of *Rhizobium*. A major obstacles in such an endeavour was to determine the complete structure of the very complex O-antigen. One of the major breakthroughs we made was the elucidation of the complete structure of the O-antigen of *R. leguminosarum* bv *trifolii* strain 4S.

We experimented with several model systems for looking at the mechanisms by which bacteria adapt to low pH. Part of the rationale for doing this was a desire to understand (or at least to be

able to rationalize) the changes in membrane and cell surface chemistry we see when bacteria live intracellularly in the plant. The role of the very long fatty acids which we first described in the membranes of *Rhizobium* are of special significance here from the standpoint of membrane stability. We have been conducting studies primarily using bacteria which are adapted to low pH. The path we took was a highly multidisciplinary, yet highly structured, one aimed at integrating our knowledge on rhizobial surface carbohydrate chemistry with emerging models and newer findings on the *Rhizobium* / legume symbiosis. It provided a detailed chemical and biochemical forum to bridge the gaps in our understanding as newer information emerged. It was intended to reconcile the various (sometimes divergent) models which wax and wane in popular opinion, using the relatively dispassionate basis of rigorous structural chemistry and biochemistry. It is our belief that, at worst, such an approach will result in a comprehensive enough body of sound science to give a rational basis on which we can reject our most popular models and embrace newer ones. In such an event our gain would be incremental but, given the complexity of the problem, very significant. At best, such an approach can point unerringly at the true causes for the phenomena that we study. This would represent an absolute gain. In either event, we are assured of moving in the right direction. The results of our research combined with that of others allows us to enunciate six very important principles.

(1) *The cell surface chemistry of Rhizobium is a critical compatibility factor with the host plant and biological, biochemical or environmental factors that perturb it either directly or indirectly will influence the outcome of the infection and nodulation process.*

(2) *The biosyntheses of the various carbohydrates and glycolipids (including chitolipooligosaccharide nod factors) are inter-connected and inter-dependent and changes that affect one usually affect several.*

(3) *The ability to synthesize glycolipids such as diglycosyl diacylglycerols appears to be critical to the formation of bacteroid and ex-planta "bacteroid-like" forms of Rhizobium.*

(4) *There is no mandatory structure for the polymerized O-antigen for the bacterium to be effective in symbiosis (since R. leguminosarum* bv. *trifolii* 4S *does not have the classical O-antigen structure)*

(5) *The synthesis of a core tetrasaccharide common to all the wild-type strains of R. leguminosarum* bv. *Trifolii that we know is not necessary for the bacterium to be effective in symbiosis (since R. leguminosarum* bv. *trifolii* 4S *does not have this core component)*

(6) *The membrane phospholipid and cell surface carbohydrate chemistry of Rhizobium is finely tuned to that of the plant either to ensure compatibility at the level of structure or biosynthesis or both.*

REFERENCES

1. Leigh, J. A. and G.C. Walker. 1994 . Exopolysaccarides of rhizobium: synthesis, regulation and symbiotic function. *Trends In Genetics.* **10:** 63-67.

2.Brink, B. A., J. Miller, R. W. Carlson and K. D. Noel. 1990. Expression of *Rhizobium leguminosarum* CFN42 genes for lipopolysaccharide in strains derivedfrom different R. leguminosarum soil isolates. *J. Bacteriol.* **172:** 548-555.

3. Carlson, R.W., S. Kalembasa, D. Turowski, P. Pachori and K. D. Noel. 1987. Characterization of the lipopolysaccharide from a *Rhizobium phaseoli* mutant that is defective in infection thread development. *J. Bacteriol.* **169:** 4923-4928.

4. Noel, K. D., K. A. Vandenbosch and B. Kulpaca. 1986. Mutations in *Rhizobium phaseoli* that lead to arrested development of infection thread. *J. Bacteriol.* **168:** 1392-1401.

5. Cava, J. R., P. M. Elias, D. A. Turowski , and K.D. Noel. 1989. Rhizobium leguminosarum CFN42 genetic regions encoding lipopolysaccharide structures essential for complete nodule development on bean plants. *J. Bacteriol.* **171:** 8-15.

6. de Maagd, R. A., A. S. Rao, I. H. M. Mulders, L. G. de Roo, M. C. M. van Loosdrecht, C. A. Wijffelman, and B. J. J. Lugtenberg. 1989. Isolation and characterization of mutants of *Rhizobium leguminosarum* bv. *viciae* 248 with altered lipopolysaccharides: possible role of surface charge or hydrophobicity in bacterial release from the infection thread. *J. Bacteriol.* **171:** 1143-1150.

7. Priefer, U. B. 1989. Genes involved in lipopolysaccharide production and symbiosis are clustered on the chromosome of *Rhizobium leguminosarum* biovar *viciae* VF39. *J. Bacteriol.* **171:** 6161-6168.

8. Dazzo, F. B., G. L. Truchet, R. I. Hollingsworth, E. M. Hrabark, H. S. Pankratz, S. Philip-Hollingsworth, J. L. Salzwedel, K. Chapman, L. Appenzeller, A. Squartini, D. Gerhold and G. Orgambide. 1991. *Rhizobium* lipopolysaccahride modulates infection thread development in white clover root hairs. *J. Bacteriol.* **173:** 5371-5384.

9.Denarie, J and J. Cullimore. 1993. Lipo-Oligosaccharide Nodulation Factors: A Minireview : new class of signaling molecules mediating recognition and morphogenesis. *Cell.* **74:** 951-954.

10. Cedergren, R.A., J. Lee, K.L. Ross, and R. I. Holligsworth. 1995. Common links in the structure and cellular localization of *Rhizobium* chitolipooligosaccharides and general *Rhizobium* membrane phospholipid and glycolipid components. *Biochemistry* **34:** 4467-4477.

11. Orgambide, G., J. Lee, R. I. Hollingsworth and F.B. Dazzo. "Structurally diverse chitolipooligosaccharide nod factors accumulate primarily in membranes of wild type *Rhizobium leguminosarum* biovar *trifolii"* (1995) *Biochemistry,* 34: 3832-3840.

12. Breedveld, M.W. and K.J. Miller. 1994. Cyclic b-glucans of members of the Family *Rhizobiaceae. Microbiological Reviews* **58:**145-161.

13. Orgambide, G. G., S. Philip-Hollingsworth, R. I. Hollingsworth and F. B. Dazzo. 1994. Flavone-enhanced accumulation and symbiosis-related biological activity of a diglycosyl

diacylglycerol membrane glycolipid from *Rhizobium leguminosarum* biovar trifolii. *J. Bacteriol.* **176:**4338-4347.

14. Cedergren, R., Y. Wang, and R. I. Holligsworth. 1996. The "missing" typical *Rhixobium leguminosarum* O antigen is attached to a fatty acylated glycerol in *R. leguminosarum* bv. Trifolii 4S, a strain that also lacks the usual tetrasaccharide "core" component. *J. Bacteriol* **178:**5529-5532.

15. Aragon, V., R. Diaz, E. Moreno, and I. Moriyon. 1996. Charaterization of *Brucella abortus* and *Brucella melitensis* native haptens as outer membrane O-type polysaccharides independent from the smooth lipopolysaccaharide. *J. Bacteriol.* **178:**1070-1079.

16. Beynon, L.m., A. J. Dumanski, R. J. C. Mclean, L.L. maclean, J. C. Richard, and M.B. Perry. 1992. Capsule structure of *Proteus mirabilis* (ATCC 49565). *J. Bacteriol.* **174:** 2172-2177.

17. Kogan, G., B.Jann. And K. Jann. 1992. Structure of the Escherichia coli O:104 polysaccharide and its identity with the capsular K9 polysaccharide. *FEMS Microbiol. Lett.* 91:135-140.

18. Lopez-lara, I. M., G. Orgambide, F. B. Dazzo, J. Olivares, and N. Toro. 1993. Charaterization and symbiotic importance of acidic extracellular polysaccharides of *Rhizobium sp.* strain GRH2 isolated from acacia nodules. *J. Bacteriol.* **175:** 2826-2823.

19. Putnoky, P., G. Petrovics, A. Kereszt, E. Grosskopf, D. Cam Ha, Z. Banfalvi and A. Kondorosi. 1990. *Rhizobium meliloti* lipopolysaccharide and exopolysaccharide can have the same function in the plant-bacterium interaction. *J. Bacteriol.* **172:** 5450-5458.

20. Williams, M. N. V., R. I. Hollingsworth, P. M. Brzoska and E. R. Signer. 1990. Rhizobium meliloti chromosomal loci required for suppression of exopolysaccharide mutations by lipopolysaccharide. *J. Bacteriol.* **172:** 6596-6598.

21. Diebold, R. and K. D. Noel. 1989. *Rhizobium leguminosarum* exopolysaccharide mutants: biochemical and genetic analyses and symbiotic behavior on three hosts. *J. Bacteriol.* **171:** 4821-4830.

22. Cedergren, R. A., and R. I. Hollingsworth. 1994. Occurrence of sufoquinovosyl diacylglycerol in some members of the family *Rhizobiaceae. J. Lipid Res.* **35:** 1452-1461.

23. Tang, Y. and R. Hollingsworth. 1997 Digalactosyl diacyl glycerols, normally exclusively plant lipids, are predominant membrane components of bacteroid forms of *Bradyrhizobium japonicum Glycobiology* 7 935-942,

24. Tang, Y. and R. Hollingsworth 1997 Synthesis of di and triglycosyl diacylglycerols in bacteroid-like forms of *Bradyrhizobium japonicum*. Ph.D. Thesis, Michigan State University.

25. Wang, J. and R. I. Hollingsworth 1997 Membrane Localization of Sulfoquinovosyl Diacylglycerol and Chitolipooligosaccharide *Nod* factors in *Rhizobium meliloti* J. Wang Ph. D. Thesis, Michigan State University

26. Y. Tang and R. Hollingsworth "Regulation of Lipid Synthesis in *Bradyrhizobium*: Low oxygen Triggers Phosphatidylinositol Biosynthesis" (1998) Applied and Environmental Microbiology, 64 1963-1966.

27 Beconi-Barker, M. G. and R. I. Hollingsworth 1997 Both cell surface antigen masking and antigen switching occur as an adaptative response of *Rhizobium* to low pH Ph.D. Thesis 1997 Michigan State University

OTHER PUBLISHED WORK GENERATED DURING THIS PROJECT

V. Bhat, H. Mayer, A. Yokota, R. Hollingsworth and R.W. Carlson. "Occurrence of Lipid A variants with 27-hydroxyoctacosanoic acid in lipopolysaccharides from the *Rhizobiaceae* group." (1991) J. Bacteriol. 173:2155-2159.

R.I. Hollingsworth and D. Lill-Elghanian. "Endotoxin structure and activity: An old problem from a new perspective." (1990) Cellular and Molecular Aspects of Endotoxin Reactions. Volume 1. A. Novotny, ed., Excerpta Medica, Amsterdam, New York.

S. Philip-Hollingsworth, R.I. Hollingsworth and F.B. Dazzo. "N-Acetylglutamic Acid: an extracellular *nod* signal of *Rhizobium trifolii* ANU843 that induces root hair branching and nodule-like primordia in white clover roots". (1991) J. Biol. Chem. 266:16854-16858.

D.S. Wagner, A. Salari, D.A. Gage, J. Keykam, J. Fetter, R. Hollingsworth and J.T. Watson. "Derivatization of peptides to enhance ionization efficiency and control fragmenttion during analysis by fast atom bombardment tandem mass spectrometry". (1991) Biological Mass Spectrometry, 20:419-425.

Y. Zhang, V.B. Priefer and R.I. Hollingsworth. "Characterization of structural defects in the lipopolysaccharide of symbiotically impaired *Rhizobium leguminosarum* biovar *vicial* VF39 mutants". (1992) Carbohydrate Research, 231:261-271.

G. Orgambide, R. Hollingsworth and F. Dazzo. "Structural Characterization of a novel diglycosyldiacylglyceride from *Rhizobium trifolii* ANU843." (1993) Carbohydrate Research, 253:151-159.

Y. Zhang, R. Cedergren, T. Nieuwenhuis and R. Hollingsworth. "N,N-(2,4-dinitro)phenyloctylamine derivatives for the isolation, purification, and mass spectrometric characterization of oligosaccharides." (1993) Analytical Biochemistry, 208:363-371.

S. Jung, S.E. Lowe, R.I. Hollingsworth and J.G. Zeikus. "*Sarcina Ventriculii* synthesizes very long chain dicarboxylic acids in response to different forms of environmental stress." (1993) Journal of Biological Chemistry, 268:2828-2835.

Y. Wang and R. Hollingsworth. "Structure of the O-antigen chain of the lipopolysaccharide of *Rhizobium trifolii* 4S." (1994) Carbohydrate Research 260 305-317.

K.I. Kim and R. Hollingsworth. "Synthesis of C-glycosides of N-acetylglucosamine by direct alkylation of 2-amino-2-deoxy-2,3,4,6-tetra acetylglucopyranosyl chloride." (1994) Tetrahedron Letters 35(7):1031-1032.

Y. Wang and R. Hollingsworth. "A solvent system for the high resolution NMR spectroscopy of membrane lipids." (1994) *Analytical Biochemistry* 225: 242-251.

K.I. Kim and R. Hollingsworth. "The total synthesis of structurally non-related endotoxin inhibitors." (1994) Bioorganic and Medicinal Chemistry Letters 4(14) 1691-1696.

S. Jung, G. Zeikus and R. Hollingsworth "A new family of very long chain , -dicarboxylic acids is a major structural fatty acyl component of the membrane lipids of *Thermoanaeiobacter ethanolicus* 39E." (1994) J. Lipid Research 35: 1057-1065

S. Jung and R. Hollingsworth. "A mathematical model explaining the molecular weights and distributions of very long chain dicarboxylic acids formed during the adaptative response of *Sarcina ventriculi*." (1995) J. Theoretical Biol., 172: 121-126.

R. Hollingsworth, Y. Zhang and U. Priefer. "Structure of the Unusual Trisaccharide lipopolysaccharide component produced by a symbiotically defective mutant of *Rhizobium leguminosarum* bv *viciae*." (1994) Carbohydrate Research , 264: 271-280.

S. Jung and R. Hollingsworth. "The structures and Stereochemistry of the very long chain bifunctional alkyl species in the membrane of *Sarcina ventriculi* indicate that they are formed by tail-to-tail coupling of normal fatty acids." (1994) J. Lipid Research, 35: 1932-1945.

R. Cedergren, J. Lee, K. Ross and R. Hollingsworth. "Common links in the structure and cellular localization of *Rhizobium* chitolipooligosaccharides and general *Rhizobium* membrane components." (1995) *Biochemistry* 34: 4467-4477

Y. wang and R. Hollingsworth. "An NMR spectroscopy and molecular mechanics study of the molecular basis for the supramolecular structure of lipopolysaccharides" (1996) *Biochemistry*. 35: 5647 - 5654

S. Jung, D. Min and R. I. Hollingsworth. "A Metropolis Monte Carlo method for analysing the energetics and dynamics of lipopolysaccharide supramolecular structure and organization" (1996) *Journal of Computational Chemistry* 17: 238 - 249.

H. Gazzano-Santoro, J. B. Parent, P. J. Conlon, D. Fishwild, H. G. Kasler, S. Wu, C. Tsai, D. A. Lill-Elghanian and R. I. Hollingsworth "Characterization of the structural elements in lipid A required for binding of a recombinant bactericidal/permeability-increasing protein" (1995) *Infection and Immunity*. 63: 2201-2205

S. Philip-Hollingsworth, G. Orgambide, J. Bradford, D. Smith, R. I. Hollingsworth and F. B. Dazzo "Mutation or increased copy number of *nodE* has no effect on the spectrum of chitolipooligosaccharide *nod* factors made by *Rhizobium leguminosarum* bv *trifolii*" (1995) *J. Biol. Chem.*, 270: 20968-20977.

G. Orgambide, S. Philip-Hollingsworth, R. I. Hollingsworth and F.B. Dazzo "Flavone-enhanced accumulation and symbiosis-related biological activity of a diglycosyl diacylglycerol membrane glycolipid from *Rhizobium leguminosarum* biovar *trifolii*." (1994) *J. Bacteriol.* 176: 4338-4347.

L. R. Berube and R. I. Hollingsworth "Membrane lipid alkyl chain motional dynamics is conserved in *Sarcina ventriculi* despite pH-induced adaptative structural modifications including alkyl chain tail to tail coupling." (1995) *Biochemistry* 34: 12005-12011.

J. Lee and R. I. Hollingsworth "Isolation and characterization of a -1-O-acyl- -1,2-diglucosyl glycoside from the membranes of a gram positive bacterium *Sarcina ventriculi*." (1996) *Tetrahedron*. 52: 3873 - 3878

R. Hollingsworth "The chemical degradation of starch: Old reactions and new frontiers" (1996) *Biotechnology Annual Review* (1996) 2 281-291

R. Cedergren, Y. Wang and R. Hollingsworth "The missing typical *Rhizobium leguminosarum* O-antigen is attached to a fatty acylated glycerol in *Rhizobium trifolii* 4S, a strain that also lacks the usual tetrasaccharide core component" (1996) Journal of Bacteriology 178 5529-5532.

J. Lee and R. Hollingsworth "Oligosaccharide -glucans with unusual linkages from *Sarcina ventriculi*" (1997) *Carbohydrate Research* 304 133-141.

S. Philip-Hollingsworth, F.B. Dazzo and R. Hollingsworth "Structural requirements of *Rhizobium* chitolipooligosaccharides for uptake and bioactivity in legumes roots as revealed by synthetic analogs and fluorescent probes" (1997) J. Lipid Research 38 1299-1241.

J. Lee and R.I. Hollingsworth "Confirmation and complete NMR spectroscopy assignment of the structure of peptidoglycan from Sarcina ventriculi, a highly adaptable gram positive bacterium" (1977) Carbohydrate Research 303 103-112.