CENTER FOR PLANT AND MICROBIAL COMPLEX CARBOHYDRATES AT THE UNIVERSITY OF GEORGIA COMPLEX CARBOHYDRATE RESEARCH CENTER

[DE-FG02-93ER20097]

Progress Report for the Funding Period

November 1, 2002 – October 31, 2003

Introduction. This progress report describes the research, service, and training activities conducted with the support of the DOE center grant. The research activities are summarized in the form of reprints or abstracts of **46** papers citing support from the DOE center grant that were produced during the reporting period. These papers include those that are published, in press, submitted, or in preparation. The papers include those produced entirely by CCRC personnel and those papers representing research work conducted in collaboration with scientists at other institutions. (See **Appendix I**.)

A major component of this grant is to provide service to researchers at other academic institutions and industries located throughout the US and other parts of the world. A summary of all our service activities during the reporting period is also included with this report, including samples of poly/oligosaccharides and antibodies distributed to scientists (see **Appendix II**). A description of the three training courses held at the CCRC during 2003 is also provided, together with the names and affiliations of participants who attended the courses (see **Appendix III**).

Next funding period. During the next funding period we will continue the extensive service and training in plant/microbial carbohydrate science as described in our original proposal. We will also continue the collaborative research on all aspects of plant and microbial carbohydrate chemistry as described in this progress report.

APPENDIX I.

Papers Citing Support of the DOE Center for Plant and Microbial Complex Carbohydrates

Papers Published:

2002

Gudlavalleti, S.K., S.B. Stevens, B.L. Reuhs, and L.S. Forsberg. 2002. Sinorhizobium sp. strain NGR234 lipopolysaccharides: Structural features and comparison of LPS from uninduced and apigenin-induced cells. In: *Nitrogen Fixation: Global Perspectives, Proceedings of the 13th International Congress on Nitrogen Fixation* (T.M. Finan *et al.*, eds.), p. 525. CABI Publishing. [Abstract not available.]

Jeyaretnam, B., J. Glushka, V.S. Kumar Kolli, and R.W. Carlson. 2002. Characterization of a novel lipid-A from *Rhizobium* species Sin-1. A unique lipid-A structure that is devoid of phosphate and has a glycosyl backbone consisting of glucosamine and 2-aminogluconic acid. *J. Biol. Chem.* 277: 41802-41810. [Reprint enclosed.]

Loh, J., R.W. Carlson, W.S. York, and G. Stacey. 2002. Bradyoxetin, a unique chemical signal involved in symbiotic gene regulation. *Proc. Natl. Acad. Sci. USA* 99: 14446-14451. [Reprint enclosed.]

Marsh, M.E., A.L. Ridall, P. Azadi, and P.J. Duke. 2002. Galacturonomannan and Golgi-derived membrane linked to growth and shaping of biogenic calcite. *J. Struct. Biol.* 139: 39-45. [Reprint enclosed.]

Pessoni, R.A.B., G. Freshour, R. De Cassia, L. Figueiredo-Ribeiro, M.G. Hahn, and M.R. Braga. 2002. Woronin bodies in *Penicillium janczewskii* Zaleski. *Braz. J. Microbiol.* 33: 127-130. [Abstract/reprint not available.]

Vandenplas, M.L., R.W. Carlson, B.S. Jeyaretnam, B. McNeill, M.H. Barton, N. Norton, T.F. Murray, and J.N. Moore. 2002. *Rhizobium* Sin-1 lipopolysaccharide (LPS) prevents enteric LPS-induced cytokine production. *J. Biol. Chem.* 277: 41811-41816. [Reprint enclosed.]

Ye, Z.-H., G. Freshour, M.G. Hahn, D.H. Burk, and R. Zhong. 2002. Vascular development in *Arabidopsis*. In: *International Review of Cytology: A Survey of Cell Biology* (K.W. Jeon, ed.), pp. 225-256. Academic Press, Amsterdam.

Vascular tissues, xylem, and phloem, form a continuous network throughout the plant body for transport of water, minerals, and food. Characterization of *Arabidopsis* mutants defective in various aspects of vascular formation has demonstrated that *Arabidopsis* is an ideal system for investigating the molecular mechanisms controlling vascular development. The processes affected in these mutants include initiation or division of procambium or vascular cambium, formation of continuous vascular cell files, differentiation of procambium or vascular cambium into vascular tissues, cell elongation, patterned secondary wall thickening, and biosynthesis of secondary walls. Identificaiton of the genes affected by some of these mutations has revealed essential roles in vascular development for a cytokinin receptor and several factors mediating auxin transport or signaling. Mutational studies have also identified a number of *Arabidopsis* mutants defective in leaf venation pattern or vascular tissue organization in stems. Genetic evidence suggests that the vascular tissue organization is regulated by the same positional information that determines organ polarity.

2003

Bergmann, C.W., L. Stanton, D. King, R.P. Clay, G. Kemp, R. Orlando, A. Darvill, and P. Albersheim. 2003. Recent observations on the specificity and structural conformation of the polygalacturonase-polygalacturonase inhibiting protein system. In: *Advances in Pectin and Pectinase Research* (F. Voragen, H. Schols, and R Visser, eds.), pp. 277-291. Kluwer Academic Publishers, The Netherlands. [Reprint enclosed.]

Freshour, G., C.P. Bonin, W.-D. Reiter, P. Albersheim, A.G. Darvill, and M.G. Hahn. 2003. Distribution of fucose-containing xyloglucans in cell walls of the *mur1* mutant of *Arabidopsis thaliana*. *Plant Physiol.* 131: 1576-1577. [Reprint enclosed.]

Glushka, J.N., M. Terrell, W.S. York, M.A. O'Neill, A. Gucwa, A.G. Darvill, P. Albersheim, and J.H. Prestegard. 2003. Primary structure of the 2-*O*-methyl-α-L-fucose-containing side chain of the pectic polysaccharide, rhamnogalacturonan II. *Carbohydr. Res.* 338: 341-352. [Reprint enclosed.]

Gudlavalleti, S.K. and L.S. Forsberg. 2003. Structural characterization of the lipid A component of *Sinorhizobium* sp. NGR234 rough- and smooth-form lipopolysaccharide: Demonstration that the distal amide linked acyloxyacyl residue containing the long chain fatty acid is conserved in *Rhizobium* and *Sinorhizobium* SPP. *J. Biol. Chem.* 278:3957-3968. [Reprint enclosed.]

Lawrence, M.L., M.M. Banes, P. Azadi, and B.Y. Reeks. 2003. The *Edwardsiella ictaluri* O polysaccharide biosynthesis gene cluster and the role of O polysaccharide in resistance to normal catfish serum and catfish neutrophils. *Microbiology* 149:1409-1421. [Reprint enclosed.]

O'Neill, M.A., S. Eberhard, B. Reuhs, W.-D. Reiter, T. Ishii, T. Fujiwara, P. Albersheim, and A.G. Darvill. 2003. Covalent cross-linking of primary cell wall pectic polysaccharides is required for normal plant growth. In: *Advances in Pectin and Pectinase Research* (F. Voragen, H. Schols, and R Visser, eds.), pp. 61-73. Kluwer Academic Publishers, The Netherlands. [Reprint enclosed.]

Jia, Z., Q. Qin, A.G. Darvill, and W.S. York. 2003. Structure of the xyloglucan produced by suspension-cultured tomato cells. *Carbohydr. Res.* 338: 1197-1208. [Reprint enclosed.]

Perrin, R.M., Z. Jia, T.A. Wagner, M.A. O'Neill, R. Sarria, W.S. York, N.V. Raikhel, and K. Keegstra. 2003. Analysis of xyloglucan fucosylation in Arabidopsis. *Plant Physiol.* 132: 768-778. [Reprint enclosed.]

Qin, Q., C.W. Bergmann, J.K.C. Rose, M. Saladie, K. Kolli, P. Albersheim, A.G. Darvill, and W.S. York. 2003. Characterization of a tomato protein that inhibits a xyloglucan-specific *endo*glucanase. *Plant J.* 34: 327-338. [Reprint enclosed.]

Vedam, V., E.L. Kannenberg, J.G. Haynes, D.J. Sherrier, A. Datta, and R.W. Carlson. 2003. A *Rhizobium leguminosarum* AcpXL mutant produces lipopolysaccharide lacking 27-hydroxyoctacosanoic acid. *J. Bacteriol.* 185: 1841-1850. [Reprint enclosed.]

Papers In Press

Guillaumie, F., J.D. Sterling, K.J. Jensen, O.R.T. Thomas, and D. Mohnen. 2003. Solid-supported enzymatic synthesis of pectic oligogalacturonides and their analysis by MALDI-TOF mass spectrometry. *Carbohydr. Res.* In press.

Solid-phase biosynthetic reactions, followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis (MALDI-TOF), was used to gain insight into the biosynthesis of pectin oligomers. Sepharose supports bearing long pectic oligogalacturonides (OGAs) anchored through a disulfide-containing cleavable linker, were prepared. The OGAs (degrees of polymerization of 13 and 14) were efficiently immobilized through the reducing end *via* formation of an oxime linkage. These OGA-derivatized matrices were subsequently employed in novel solid-phase enzymatic reactions, with the pectin biosynthetic enzyme, α-1,4-galacturonosyltransferase, GalAT (solubilized from *Arabidopsis thaliana*) and the glycosyl donor, uridine diphosphate-galacturonic acid (UDP-GalA). Solid-supported biosynthesis was followed by cleavage of the immobilized OGAs and direct analysis of the products released into the liquid phases by MALDI-TOF mass spectrometry. In time course studies conducted with an immobilized (α-D-GalA)$_{14}$ and limiting amounts of the glycosyl donor, the predominant product was an OGA extended by one GalA residue at the non-reducing end (*i.e*. (GalA)$_{15}$). When UDP-GalA was added in $\approx$ 5-fold excess compared to immobilized (GalA)$_{13}$, OGAs up to the 16-mer were synthesized, confirming the non-processivity of the GalAT *in vitro*.

Kemp, G., C.W. Bergmann, R. Clay, A.J. Van der Westhuizen, and Z.A. Pretorius. 2003. Isolation of a polygalacturonase-inhibiting protein (PGIP) from wheat. *Mol. Plant-Microbe Interact.* In press.

Evidence for the presence of a polygalacturonase-inhibiting protein (PGIP) from a monocotyledonous cereal is presented. A 40.3-kDa PGIP that was closely associated with the cell wall was acetone-extracted and purified from wheat (*Triticum aestivum* L.) leaves and stems. Wheat PGIP exhibited a highly selective inhibitory activity against endopolygalacturonase (EPG) from various fungi. Of nine EPG tested, wheat PGIP only inhibited EPG from *Cochliobolus sativus*, a pathogen of the tribe Poaceae. A short N-terminal amino acid sequence of wheat PGIP shows no similarity to any other characterized PGIP.

Kolli V.S.K., J. Johnson, R. Orlando, A.G. Darvill, P. Albersheim, and S.-C. Wu. 2003. Proteomic identification of extracellular proteins secreted by the rice blast fungus. In: *Proceeding of ASMS 2003 (American Society of Mass Spectrometry)*. In press.

Fungi secrete a wide array of different proteins as a response to their environment in which the fungi are growing. The extracellular proteins (ECPs) secreted by fungi perform diverse functions, e.g assimilation of nutrients, communication and interactions between pathogenic fungi and their host. It is noteworthy that most proteins known to function as pathogenic and avirulance factors are extracellular proteins. Despite their importance, however, there has been no systemic study on ECPs from a fungal plant pathogen. On the other hand, the wealth of sequence information in the genomics era has brought rapid technological innovations towards high-throughput functional analysis of genes and their proteins.

Magnaporthe grisea, the fungus that causes rice blast disease, is one of the main pathological threats to food supplies worldwide. Annually, enough rice is lost to this disease to feed 60 million people per year. To date, this disease has presence in more than 85 countries in the world. Rice blast is a spot disease with initial symptoms of white to gray-green lesions produced on all parts of shoot. The infection cycle starts with the germination of spore on a host leaf and passes through several stages during which the pathogen will respond to several cues from the plant. The biology of host-pathogen interactions is very complex, and the knowledge of molecular movement during infection is essential to understand the disease process for better disease control. At the genomic level, the nearly finished *M. grisea* sequencing project is paving the way for speedy characterization of the genes involved in fungus-plant interactions. This study is sought to identify *M. grisea* ECPs expressed under various physiological growth conditions by a high throughput proteomics approach, with an emphasis on proteins secreted during infection.

Matsunaga, T., T. Ishii, S. Matsumoto, M. Higuchi, A. Darvill, P. Albersheim, and M.A. O'Neill. 2003. Occurrence of the primary cell wall polysaccharide rhamnogalacturonan II in Pteridophytes, Lycophytes, and Bryophytes: Implications for the evolution of vascular plants. *Plant Physiol.* In press.

Borate ester cross-linking of the cell wall pectic polysaccharide rhamnogalacturonan II (RG-II) is required for the growth and development of angiosperms and gymnosperms. Here, we report that the amounts of borate cross-linked RG-II present in the primary walls of members of the most primitive extant vascular plant groups (Lycopsida, Filicopsida, Equisetopsida, and Psilopsida) are comparable to the amounts of RG-II in the primary walls of angiosperms. By contrast, the members of the avascular bryophytes (Bryopsida, Hepaticopsida, and Anthocerotopsida) have primary walls that contain small amounts (approximately 1% of the amounts of RG-II present in angiosperm walls) of an RG-II-like polysaccharide. The glycosyl sequence of RG-II is conserved in vascular plants, but these RG-IIs are not identical as the non-reducing L-rhamnosyl residue present on the aceric acid-containing side chain of RG-II of all previously studied plants is replaced by a 3-*O*-methyl rhamnosyl residue in the RG-IIs isolated from *Lycopodium tristachyum*, *Ceratopteris thalictroides*, *Platycerium bifurcatum*, and *Psilotum nudum*. Our data indicate that the amount of RG-II incorporated into the walls of plants increased during the evolution of vascular plants from their bryophyte-like ancestors. Thus, the acquisition of a boron-dependent growth habit may be correlated with the ability of vascular plants to maintain upright growth and to form lignified secondary walls. The conserved structures of pteridophyte, lycophyte, and angiosperm RG-IIs suggests that the genes and proteins responsible for the biosynthesis of this polysaccharide appeared early in land plant evolution and that RG-II has a fundamental role in wall structure.

O'Neill, M.A., T. Ishii, P. Albersheim, and A.G. Darvill. 2003. Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Ann. Rev. Plant Biol.* (Invited review) In press.

Rhamnogalacturonan II (RG-II) is a structurally complex pectic polysaccharide that was first identified in 1978 as a quantitatively minor component of suspension-cultured sycamore cell walls. Subsequent studies have shown that RG-II is present in the primary walls of angiosperms, gymnosperms, lycophytes, and pteridophytes and that its glycosyl sequence is conserved in all vascular plants that have been examined to date. This is quite remarkable because RG-II is composed of at least twelve different glycosyl residues linked together by more than twenty different glycosidic linkages. However, only a few of the genes and proteins required for RG-II biosynthesis have been identified. The demonstration that RG-II exists in primary walls as a dimer that is covalently cross linked by a borate diester was a major advance in our understanding of the structure and function of this pectic polysaccharide. Dimer formation results in the cross linking of the two homogalacturonan chains upon which the RG-II molecules are constructed and is required for the formation of a three-dimensional pectic network *in muro*. This network contributes to the mechanical properties of the primary wall and is required for normal plant growth and development. Indeed, changes in wall properties that result from decreased borate cross-linking of pectin may lead to many of the symptoms associated with boron deficiency in plants.

Ramírez-Coronel, M.A., G.V. González, A. Darvill, and C. Augur. 2003. A novel tannase from *Aspergillus* niger with β-glucosidase activity. *Microbiology*. In press. [Preprint enclosed.]

O'Neill, M.A. and W.S. York. 2003. The composition and structure of plant primary cell walls. In: *The Plant Cell Wall* (J.K.C. Rose, ed.). Blackwell Publishing, Sheffield, United Kingdom. In press. [Abstract not available.]

Wu, S.-C., J. Johnson, A.G. Darvill, P. Albersheim, and R. Orlando. 2003. Proteomics of *Magnaporthe grisea*: liquid chromatography mass spectrometry for the identification of extracellular proteins. In: *Rice Blast Disease*, Proceedings of the Third International Rice Blast Conference. In press.

The recent wealth of sequence information from a broad spectrum of prokaryotic and eukaryotic genomes has brought rapid technological innovations towards high-throughput functional analysis of whole genomes. Functional genomics addresses expression and function of all genes with regard to cell type, organ and physiological state. One branch of functional genomics is the study of proteins, using proteomics technology. Proteomics is the study of the complete protein component, or a proteome, of an organism. The proteome is highly dynamic. For example, the presence of proteins, their abundance, state of modification and sub-cellular location, are all affected by the physiological state of the cell or tissue. It is estimated that, depending on the species, as many as a few thousand to ten thousand proteins may be present in a cell at a given moment. Proteomics was developed with the realization of this dynamic complexity of a proteome, as well as the tremendous discrepancy among genes predicted from a whole-genome sequence, transcripts detected using DNA microarray assays, and proteins actually present in an organism's particular state, organs or cells.

Traditional proteomics involves identification by mass spectrometry of proteins separated by two-dimensional gel electrophoresis (2DE). Using this technology, gel plugs containing protein 'spots' from 2DE are treated with an endoprotease (e. g. trypsin), and the released peptides eluted from each of the gel plugs analyzed by Matrix-Assisted Laser Desorption/Ionization mass spectrometry (MALDI MS), or electrospray mass spectrometry (ES-MS). The mass spectra of the peptides, or MS fingerprints, are then queried against a non-redundant sequence database that contains lists of molecular weights of peptides predicted from proteolysis of all known or theoretical protein sequences. To increase query specificity, an aliquot of the digested peptide sample can also be subjected to tandem mass spectrometry (MS/MS) on a Quadrupole/Time-of-Flight (Q-Tof) mass spectrometer system to obtain specific amino acid sequences from a few of the peptides present in the sample. The amino acid sequence and the mass of a peptide in the sample can then be combined into what is commonly called a 'Peptide Sequence Tag' or PST, which can be used to query against a sequence database resulting in great specificity.

York, W.S., Q. Qin, and J.K.C. Rose. 2003. Proteinaceous inhibitors of endo-β-glucanases. *Biochem. Biophys. Acta*. In press.

Both plants and filamentous phytopathogens secrete proteins that inhibit *endo*-β-glucanases. The first *endo*-β-glucanase inhibitor proteins to be discovered are XEGIP, a tomato protein that inhibits fungal xyloglucan specific *endo*-β-1,4-glucanases, and GIP1, an oomycete protein that inhibits *endo*-β-1,3-glucanases produced by the plant host. These inhibitor proteins act by forming high-affinity complexes with their *endo*glucanase ligands. A family of XEGIP-like proteins has been identified. At least one member of this family (extracellular dermal glycoprotein, EDGP) has been shown to have *endo*glucanase-inhibitor activity, while other members have sequence similarity to a xylanase inhibitor from wheat (TAXI-1). The oomycete inhibitor GIP1 is a catalytically inactive serine protease homolog (SPH) whose structure is unrelated to XEGIP. Both types of inhibitor proteins are likely to affect the interactions of plants with filamentous phytopathogens, and a basic model describing their roles in pathogenesis is proposed.

<u>Papers Submitted</u>

D'Haeze, W., J. Glushka, R. De Rycke, M. Holsters, and R.W. Carlson. 2003. Structural characterization of extracellular polysaccharides synthesized by the micro-symbiont *Azorhizobium caulinodans* and elucidation of its role during early stages of nodule invasion on the tropical legume *Sesbania rostrata*. Submitted.

Azorhizobium caulinodans, a micro-symbiont of *Sesbania rostrata*, enters its host plant via the formation of outer cortical infection pockets, a process which is characterized by a massive production of H_2O_2. Infection threads guide bacteria from infection pockets towards nodule primordia. Previously, two mutants were constructed that produce similar types of lipopolysaccharides (LPS) to one another but different from the parental LPS, and are affected in the production of extracellular polysaccharides (EPS). One was blocked at the infection pocket stage, and the other affected in the release from infection threads. The latter mutant was surrounded by a thin layer of EPS when present in infection pockets in contrast to the wild-type strain that produced massive amounts of EPS. The former mutant was unable to produce EPS suggesting a role of EPS in protecting the micro-symbiont against H_2O_2. Structural characterization of EPS purified from cultured and nodule bacteria revealed that these were linear homopolymers of α-1,3-linked 4,6-O-(1-carboxyethylidene)-D-galactosyl residues. In situ H_2O_2 localization demonstrated that an increased EPS production during early stages of invasion prevents the incorporation of H_2O_2 into the interior of the bacteria. In addition, a set of ex planta assays confirmed this positive correlation between increased EPS production and enhanced protection against H_2O_2.

Ferguson, G.P., A. Datta, J. Baumgartner, R.M. Roop II, R.W. Carlson, and G. C. Walker. 2003. Adrenoleukodystrophy similarity reveals *sinorhizobia* and *brucellae* BacA affect Lipid A acylation. Submitted

Sinorhizobium meliloti, a legume symbiont, and *Brucella abortus*, a phylogenetically related mammalian pathogen, both require BacA to establish chronic intracellular infections in their respective hosts. We show that BacA is related to the human Adrenoleukodystrophy protein, defects in which result in a neurodegenerative disorder due to reduced β-oxidation of very-long-chain fatty acids (VLCFA, C>22) by peroxisomes. This insight led us to discover that BacA affects the VLCFA (27-OHC28:0 and 29-OHC30:0) content of both sinorhizobia and brucellae lipid A. Models are discussed for how this lipid A alteration could affect the ability of sinorhizobia and brucellae to establish chronic infections.

Forsberg, L.S., K.D. Noel, J. Box, and R.W. Carlson. 2003. Genetic locus and structural characterization of the biochemical defect in the O-antigenic polysaccharide of the symbiotically deficient *Rhiozobium etli* mutant, CE166: Replacement of N-acetylquinovosamine with its hexosyl-4-ulose precursor. Submitted.

The O-antigen polysaccharide (OPS) of *Rhizobium etli* CE3 LPS is linked to the core oligosaccharide region *via* an N-acetylquinovosaminosyl (QuiNAc) residue. A mutant of CE3, strain CE166, produces an LPS with a reduced level of OPS, and a suppressed mutant, CE166α, produces LPS with near normal OPS levels even though both mutants are deficient in QuiNAc production. Isolation and characterization of the OPS from CE166 and CE166α showed that the QuiNAc residue was replaced by its 4-keto derivative, 2-acetamido-2,6-dideoxy-hexosyl-4-ulose. The identity of this residue was determined by NMR and mass spectrometry of the OPS and by GC-MS analysis of the 2-acetamido-4-deutero-2,6-dideoxy-hexosyl derivatives produced by reduction of the 4-keto group using borodeuteride. Mass spectrometric and methylation analyses revealed that the 2-acetamido-2,6-dideoxy-hexosyl-4-ulose residue was 3-linked and was attached to the core-region external Kdo III residue of the LPS; the same position as that of QuiNAc in the LPS from the CE3 parent. DNA sequence analysis revealed that the transposon insertion in strain CE166 was located in an open reading frame whose predicted translation product, LpsQ, shows greatest sequence similarity to WbpV of *Pseudomonas aeruginosa* serotypes O5 and O6. Thus, both chemical and the sequence analyses support the conclusion that *lpsQ* and *wbpV* encode UDP-2-acetamido-2,6-dideoxy-hexosyl-4-ulose reductase, the second step in the synthesis of UDP-QuiNAc from UDP-GlcNAc.

Laus, M.C., T. J. Logman, A. A. N. van Brussel, M.-Y. Gao, P. Azadi, R. W. Carlson, and J. W. Kijne. 2003. Identification of a UDP-glucose dehydrogenase, exo5, involved in the production of surface polysaccharides in *Rhizobium leguminosarum* and its role in the nodulation process of *Vicia sativa* ssp. *nigra*. Submitted.

Analysis of two exopolysaccharide-deficient mutants of *Rhizobium leguminosarum*, RBL5808 and RBL5812, revealed independent Tn5 transposon integrations in a single gene, called *exo5*. Based on structural and functional homology, this gene has been identified as a UDP-glucose dehydrogenase responsible for the oxidation of UDP-glucose to UDP-glucuronic acid. A mutation in the *exo5* gene yields bacteria which lack UDP-glucuronic acid, hereby affecting all glucuronic acid and, consequently, galacturonic acid containing polysaccharides. Exo5-deficient rhizobia do not produce extracellular polysaccharide (EPS) and capsular polysaccharide (CPS), carbohydrate composition analysis and NMR studies demonstrated that EPS and CPS are of similar nature.

Lipopolysaccharide (LPS) molecules are deficient for galacturonic acid, normally present in the core of LPS. Sensitivity of *exo5* mutant rhizobia for hydrophobic compounds showed the involvement of these galacturonic acid moieties in outer membrane structure. Nodulation studies on *Vicia sativa* ssp. *nigra* showed that *exo5* mutant rhizobia are impaired in successful infection thread colonization. Root infection could be restored by simultaneous inoculation with a Nod factor-defective strain which retained the ability to produce EPS/CPS. However, colonization of the nodule tissue was impaired.

Lerouge, I., C. Verreth, J. Michiels, R.W. Carlson, A. Datta, M.Y. Gao, and J. Vanderleyden. 2003. Three genes encoding for putative methyl and acetyltransferases map adjacent to the *WZM* and *WZT* genes and are essential for *O*-antigen biosynthesis in *Rhizobium-etli* CE3. Submitted.

The recent elucidation of the structure of the O-antigen of *Rhizobium etli* CE3 (Forsber*g* et al. 2000) predicts that the *R. etli* CE3 genome must contain genes encoding acetyl- and methyltransferases to confer the corresponding modifications to the O-antigen. We identified three open reading frames upstream of *wzm*, encoding the membrane component of the O-antigen transporter and located in the *lps*α-region of *Rhizobium etli* CE3. The *orf*s encode respectively two putative acetyltransferases with similarity to the CysE-LacA-LpxA-NodL family of acetyltransferases and one putative methyltransferase with sequence motifs common to a wide range of S-adenosyl-L-methionine-dependent methyltransferases. Mutational analysis of the *orf*s encoding the putative acetyltransferases and methyltransferase revealed that the acetyl and methyl decorations mediated by these specific enzymes are essential for O-antigen synthesis. Composition analysis and HPAEC analysis of the LPSs of the mutants show that all of these LPSs contain an intact core region and lack the O-antigen polysaccharide. The possible role of these transferases in the decoration of the O-antigen of *R. etli* is discussed.

Reuhs, B.L., J. Glenn, S.B. Stephens, J.S. Kim, D.B. Christie, J. Glushka, E. Zablackis, P. Albersheim, A.G. Darvill, and M.A. O'Neill. 2003. L-Galactose replaces L-fucose in the pectic polysaccharide rhamnogalacturonan II synthesized by the L-fucose-deficient *mur1* Arabidopsis mutant. Submitted.

Arabidopsis thaliana mur1 is a dwarf mutant with altered cell-wall properties, in which L-fucose is partially replaced by L-galactose in the xyloglucan and glycoproteins. We found that the *mur1* mutation also affects the primary structure of the pectic polysaccharide rhamnogalacturonan II (RG-II). In *mur1* RG-II a non-reducing terminal 2-*O*-methyl L-galactosyl residue and a 3,4-linked L-galactosyl residue replace the non-reducing terminal 2-*O*-methyl L-fucosyl residue and the 3,4-linked L-fucosyl residue, respectively, that are present in wild-type RG-II. Furthermore, we found that a terminal non-reducing L-galactosyl residue, rather than the previously reported D-galactosyl residue, is present on the 2-*O*-methyl xylose-containing side chain of RG-II in both wild type and *mur1* plants. Approximately 95% of the RG-II from wild type and *mur1* plants is solubilized as a high molecular weight (>100KDa) complex, by treating walls with aqueous potassium phosphate. The molecular mass of RG-II in this complex was reduced to 5 – 10 kDa by treatment with endopolygalacturonase, providing additional evidence that RG-II is covalently linked to homogalacturonan. The results of this study provide additional information on the structure of RG-II and the role of this pectic polysaccharide in the plant cell wall.

Reuhs, B.L., B. Relic, C. Marie, T. Ojanen-Reuhs, S..B. Stephens, L.S. Forsberg, C.-H. Wong, S. Jabbouri, and W.J. Broughton. 2003. Phase-shift and chronic infection of *Vigna unguiculata* by *Rhizobium* sp. NGR234 require a *Pseudomonas aeruginosa* A-band like O-antigen. Submitted.

Rhizobium sp. NGR234 contains three replicons, the smallest of which (pNGR234a) carries many symbiotic genes, including those required for nodulation and Nod-factor biosynthesis. Activation of nod-gene expression depends on plant-derived flavonoids, NodD transcriptional activators and nod-box promoter elements. Nod-boxes NB6 and NB7 delimit six different types of genes, one of which (fixF) results in the formation of ineffective nodules on *Vigna unguiculata* when mutated. Wild-type NGR234 produces a distinct, flavonoid-inducible polysaccharide that is not produced by the mutant (NGR(fixF). This polysaccharide is also found in nitrogen-fixing bacteroids isolated from *V. unguiculata*. Electron microscopy showed that peribacteroid membrane formation is perturbed in nodule cells infected by the fixF mutant, indicating that it is required for the bacterial phase-shift from free-living to bacteroid cells. Accordingly, polysaccharides were purified from free-living cells cultured in the presence of apigenin.

Structural analyses showed that they are specialized O-antigens attached to a modified lipopolysaccharide (LPS) core. The primary sequence of the O-antigen is [-3)-(-L-Rhap-(1,3)-(-L-Rhap-(1,2)-(-L-Rhap-(1-]n, and the LPS core lacks the acidic sugars associated with the antigenic outer core. This rhamnan has the same primary sequence as the A-band O-antigen of *Pseudomonas aeruginosa*, except that the A-band polysaccharide is composed of D-rhamnose. A-band LPS is selectively maintained on the *P. aeruginosa* cell surface during chronic cystic fibrosis lung infections, where it is associated with an increased duration of infection. Similarly, the presence of the rhamnan in NGR234 bacteroids is correlated with the chronic rhizobial infection required for nodule function.

Tzeng, Y.-L., A. Datta, K. Ambrose, J. K. Davies, R. W. Carlson, D. S. Stephens, and C..M. Kahler. 2003. The MisR/MisS two-component regulatory system modifies inner core structure of lipooligosaccharide in *Neisseria Meningitidis.* Submitted.

Lipooligosaccharide (LOS, endotoxin) of *Neisseria meningitidis* is the major inflammatory mediator that contributes to the pathogenesis of meningococcal septicemia and meningitis. LOS is composed of a symmetrically acylated lipid A moiety; a conserved inner core of two 3-deoxy-*D*-manno-octulosonic acid (Kdo), two heptose (Hep) sugars, and an N-acetyl glucosamine; and an oligosaccharide α-chain of variable length. In combination with the α-chain heterogeneity, variable attachments to the HepII residue of the inner core result in immunologically distinct LOS structures: immunotypes L1 through L12. Lpt-3, the O-3 phosphoethanolamine (PEA) transferase, and LgtG, the O-3 glucosyltransferase, mediate the substitution of PEA or glucose (Glc) at the O-3 position of HepII in L3 and L2 LOS immunotypes respectively. Structural characterization of LOS purified from a mutant containing an erythromycin insertion in the response regulator of a two-component regulatory system, named *misR/misS* for meningococcal inner core structure, by a combination of methylation, MS, and NMR analyses revealed the loss of all PEA decorations on the LOS inner core of *N. meningitidis* strain NMB that expresses a mixture of L2 and L3 immunotype LOS structures. The O-3 position of HepII was completely substituted with glucose, indicating that the *misR* mutant produced L2 structure exclusively. Inactivation of *lgtG* in strain NMB, as expected, resulted in an LOS inner core without glucose, but these structures also lacked the O-3 linked PEA even though Lpt-3 remained intact. Consistently, a double mutation of *lgtG* and *misR* in strain NMB yielded a LOS structure where no PEA or Glc substitution of HepII was detected. Quantitative real time PCR experiments revealed increased expression of *lgtG* in the *misR* mutant. No change was observed for *lpt-3* transcription. The absence of PEA substitution in the *misR* and *lgtG* mutants was likely the result of an as yet unknown PEA hydrolase(s) being activated in these mutants. These data also indicated a novel pathway for the regulation of LOS inner core remodeling in *N. meningitidis* through the MisR/MisS environmental sensing two-component regulatory system.

Vedam, V., J.G. Haynes, E. Kannenberg, R.W. Carlson, D.J. Sherrier. 2003. A *Rhizobium leguminosarum* lipopolysaccharide Lipid-A mutant induces nitrogen-fixing nodules with delayed and defective bacteroid formation. Submitted.

LPS from pea nodulating strain *Rhizobium leguminosarum* bv. *viciae* 3841, as all other members of the *Rhizobiaceae* contains a very long chain fatty acid; 27-hydroxyoctacosanoic acid (27OHC28:0) in its lipid A region. The exact function and importance of this residue, however is not known. In this work, a previously constructed mutant, *Rhizobium leguminosarum* bv. *viciae* 22, deficient in the fatty acid residue was analyzed for symbiotic phenotype. While the mutant was able to form nitrogen fixing nodules, a detailed study of the timing and efficiency of nodulation using light and electron microscopy showed that there was a delay in the onset of nodulation and nodule tissue invasion. Further, microscopy showed that the mutant was unable to differentiate normally forming numerous irregularly shaped bacteroids, that the resultant mature bacteroids were unusually large, and that several bacteroids were frequently enclosed in a single symbiosome membrane, a feature not observed in parent bacteroids. In addition, the mutant nodules were delayed in the onset of nitrogenase production and showed reduced nitrogenase throughout the testing period. These results imply that the lack of 27OHC28:0 in the lipid A in mutant bacteroids results in altered membrane properties that are essential for the development of normal bacteroids.

Watt, G., C. Leoff, A.D. Harper, and M. Bar-Peled. 2003. RmlC and RmlD prokaryotic genes evolved to one bifunctional plant protein: Purification, characterization and cloning of *Arabidopsis* 3,5-epimerase/4-keto-reductase, a T/UDP-Rha synthase. Submitted.

L-Rhamnose is an essential sugar found in numerous plant molecules ranging from cell wall pectic polysaccharides to diverse secondary metabolites including toxic and colorful flower anthocyanins. Synthesis of UDP-rhamnose or TDP-rhamnose in plants from T/UDP-4-keto-6-deoxy-Glc involves 3,5-epimerization and 4-keto-reduction, which in bacteria is mediated by two gene products of rmlC and rmlD, respectively. However, the enzymes involved in these steps were not purified in plants. Using the amino-acid sequences from corresponding prokaryotic genes, rmlC and rmlD, we identified one plant gene containing similar structural features to both prokaryotic genes. Therefore, we cloned an *Arabidopsis* gene that was highly expressed in all tissue examined and encoded a ~33.5 kDa protein. This recombinant enzyme was found to convert TDP or UDP-4-keto-6-deoxy-Glc to respective TDP- and UDP-rhamnose in the presence of NADPH. With respect to the prokaryotic enzymes, the plant enzyme possesses dual activities, specifically epimerase and reductase, and was therefore named T/URS for T/UDP-rhamnose synthase. T/URS has maximum activity at pH 5.5-7.5 and at temperature 30°C. The apparent Km for NADPH is 90 μM and 16.9 μM for TDP-4-keto-6-deoxy-Glc. This is the first described example of sugar nucleotide synthesis where two prokaryotic gene products possess the same activities as a bifunctional plant gene product.

Zughaier, S.M., Y.-L. Tzeng, S. M. Zimmer, A. Datta, R. W. Carlson, and D.S. Stephens. 2003. Activation of the macrophage CD14/TLR4 pathway by *Neisseria meningitidis* lipooligosaccharide: the importance of 3-Deoxy-D-manno-octulosonic acid (KDO) linked to Lipid A. Submitted.

Meningococcal lipopoly(oligo)saccharide (LOS) is a major inflammatory mediator of fulminant meningococcal sepsis and meningitis. Highly purified wild type meningococcal LOS and LOS from genetically-defined mutants of *Neisseria meningitidis* that contained specific mutations in LOS biosynthesis pathways were used to confirm that meningococcal LOS activation of macrophages was CD14/TLR4-MD-2 dependent and to elucidate the LOS structural requirement for TLR4 activation. Expression of TLR4 but not TLR2 was required and antibodies to both TLR4 and CD14 blocked meningococcal LOS activation of macrophages. Equal molar amounts of meningococcal LOS consistently yielded two fold or greater cytokine, nitric oxide and ROS release than *E. coli* LPS ($p<0.0001$). Meningococcal LOS α or β chain oligosaccharide structure did not influence CD14/TLR4-MD-2 activation. However, lipid A, expressed by meningococci with defects in KDO biosynthesis or transfer, resulted in a ~10 fold ($p<0.0001$) reduction in biologic activity compared to KDO2 containing meningococcal LOS. Removal of KDO2 by acid hydrolysis also dramatically attenuated cellular responses. Competitive inhibition assays showed similar binding of glycosylated and unglycosylated lipid A to CD14/TLR4-MD-2. Decrease in the number of lipid A phosphate head groups or penta-acylated meningococcal LOS modestly attenuated biologic activity. Meningococcal endotoxin is a potent agonist of the macrophage CD14/TLR4-MD-2 receptor, helping explain the fulminant presentation of meningococcal sepsis and meningitis. KDO2 linked to meningococcal lipid A was structurally required for maximal activation of the TLR4 pathway and indicates an important role for KDO-lipid A in endotoxin biologic activity.

Papers In Preparation

Freshour, G., M.R. Braga, J.W. Schiefelbein, P. Albersheim, A.G. Darvill, and M.G. Hahn. 2003. Developmentally-regulated insertion of a rhamnogalacturonan I eitope in *Arabidopsis thaliana* roots. In preparation.

A monoclonal antibody, CCRC-M2, specific for an epitope on the pectic polysaccharide, rhamnogalacturonan I, was used to study the dynamics of cell wall polysaccharides in developing seedlings of *Arabidopsis thaliana*. Previous work showed that the epitope recognized by CCRC-M2 is absent from walls at the root apex of wild-type plants. This epitope first appears in the walls of epidermal cells (atrichoblasts) that do not form root hairs, and appears later in root development in the walls of root-hair-forming epidermal cells (trichoblasts) and in those of the cortex. In mature root tissue, only the epidermal and cortical walls are labeled. These studies were extended to mutants that are altered in their pattern of root hair formation. In mutants that make no or only small numbers of root hairs (R-1439, *rhd6*), labeling with CCRC-M2 is observed initially in all epidermal cells, with the entire cortex labeling at a later stage in development. Treatment of *rhd6* with 10 nM auxin results in increased production of root hairs, resulting in a CCRC-M2 labeling pattern that corresponded to that observed in wild-type plants. In mutants that produce ectopic root hairs (*ttg*, *gl2*), only a few epidermal cells were initially labeled. Serial sectioning of the root demonstrated that the labeled epidermal cells do not produce a root hair, while all unlabeled, epidermal cells at this developmental stage produce root hairs. At a later stage in development, CCRC-M2 labeling appears in the trichoblasts and cortical cells. In the mature portions of the roots of all of these mutants, CCRC-M2 labeling was identical to that observed in mature wild-type roots. Our results confirm that cell wall polysaccharide epitopes are regulated in a cell type- and developmental stage-specific manner.

Johnson, J., M. Xie, H.C.M. Kester, J.A.E. Benen, J. Visser, R. Orlando, and C. Bergmann. 2003. Tandem mass spectrometric analysis of mutant *Aspergillus niger* pectin methylesterase. In preparation. [Abstract not available.]

Kim, Y.H., M. Xie, R. Orlando, C. Bergmann, and K. Kolli. 2003. Characterization of N-linked glycans of the recombinant *endo*polygalacturonases A and C from *Aspergillus niger*. In preparation.

*Endo*polygalacturonase A (PGA) and *endo*polygalacturonase C (PGC), secreted by *Aspergillus niger*, are two pectin degrading enzymes (PDEs) involved in degradation of plant cell-wall materials through their hydrolysis of the homogalacturonan part of the pectin network [Pařenicová *et al.*, 2000]. PDEs are important during pathogenesis and also have a number of industrial uses, including wide application in the food industry [Sakai *et al.*, 1993; Cooper, 1993]. Among these PDEs, PGA and PGC are of interest because they, and PGB, are constitutively expressed, while the major expressed PGs must be induced by the presence of pectin [Pařenicová *et al.*, 2000]. Thus, PGA, PGC, and PGB are likely required during the early stages of pathogenicity, and thus are of critical importance to successful attack. Many of the PDEs produced by fungi have been identified as being glycosylated [Archer and Peberdy, 1997; Colangelo *et al.*, 1999; Yang *et al.*, 1997]. The glycosylation state of the various PDEs may impact pathogenesis; however, the effects of the carbohydrate side chains on the properties of PDEs are not known. A thorough understanding of the glycosylation of each PDE is essential when such enzymes are overexpressed for both industrial and basic research applications. The various conditions and hosts that are chosen for overexpression may induce variation in the post-translational modifications of the recombinant proteins. Therefore, the resulting enzymes must be characterized to ensure the validation of the product of overexpression [Archer and Peberdy].

Stanton, L., C.W. Bergmann, R.P. Clay, P. Albersheim, and A. Darvill. 2003. Polygalacturonase-inhibiting proteins can function as activators. In preparation.

The interaction between fungal endopolygalacturonases (EPGs) and polygalacturonase inhibiting proteins (PGIPs) found in plant cell walls has been well established. Studies of these pectin degrading enzymes and their corresponding PGIPs have demonstrated that the typical EPG/PGIP interaction is characterized by high-affinity, reversibility, and a one:one stoichiometry. The interaction typically results in an effectively lowering of the catalytic rate of a particular endopolygalacturonase by up to 99.7%. Various EPG and PGIP isoforms and/or glycoforms have been isolated and characterized. Different combinations of EPGs and PGIPs have been shown to demonstrate a variety of degrees of enzyme inhibition. The complexity resulting from the wide range of EPG/PGIP interactions has prompted many researchers to suspect the involvement of these proteins in the production of specific signals (oligosaccharins) during plant pathogenesis. Recent studies in our laboratory have indicated that for certain combinations of PGIP isoforms with specific EPGs, the level of catalysis by the EPG is increased beyond that characteristic of the enzyme alone. This represents activation or enhancement, rather that inhibition of EPG, suggesting the need for a re-evaluation of the conventional description applied to PGIPs; suggestions include 'Polygalacturonase-Binding Protein' and 'Polygalacturonase-Modulating Protein'.

Tiné, M.A.S., M.R. Braga, G. Freshour, M.G. Hahn, and M.S. Buckeridge. 2003. Detection and subcellular localization of fucosylated and non-fucosylated seed xyloglucan in storage cell walls of Leguminous seeds. In preparation.

Many plants synthesize and accumulate storage xyloglucans in their seeds during seed maturation and then mobilize these polysaccharide reserves during seed germination. The structures of such storage xyloglucans usually differ from those of the structural xyloglucans characteristic of primary cell walls in plants. During structural studies of the storage xyloglucan synthesized by the tropical legume, *Hymenaea courbaril*, we detected the presence of small amounts of fucosylated primary wall xyloglucan. We have now used the monoclonal antibody, CCRC-M1, which specifically recognizes a fucose-containing epitope found in many xyloglucans, to localize fucosylated xyloglucans in *H. courbaril* seeds during maturation and germination. In mature seeds, fucosylated xyloglucan is present in two narrow wall domains, one inner layer near the plasma membrane and one outer layer near the middle lamella. The larger wall domain located between these two domains appears to contain the non-fucosylated storage xyloglucan, which does not label with CCRC-M1, but does stain with iodine. During seed maturation, the domains are laid down sequentially; first a layer of fucosylated xyloglucan, followed by the layer containing the storage xyloglucans, and finally a second layer of fucosylated xyloglucan. During seed germination, only the central wall domain containing the storage xyloglucans is degraded. These results highlight the rather complex topology of structurally different xyloglucans within the wall. Our results also suggest that fucosylation of xyloglucan is carefully regulated during storage wall biosynthesis and that storage mobilization must be finely controlled to selectively mobilize only the appropriate polysaccharide(s) within storage tissues.

Wu, S.-C., J.E. Halley, A.G. Darvill, and P. Albersheim. 2003. Identification of a *Magnaporthe grisea endo*-β-1,4-D-xylanase by gene knockout analysis: Protein purification and heterologous expression. In preparation.

The ascomycetous fungus, *Magnaporthe grisea*, is a destructive pathogen of rice. We are attempting to determine whether enzymes secreted by *M. grisea* can fragment polysaccharide components of rice cell walls, and whether the host plant can detect and respond to its own wall polysaccharide fragments by activating its defenses against microbes. Toward those goals, we analyzed a pool of expressed sequence tags (ESTs) from *M. grisea* that were grown in a culture medium with rice cell walls as the sole carbon source. One of the ESTs was found to encode a protein similar to Group 10 *endo*-β-1,4-D-glycanases with a putative N-terminal Class III (‘fungal’) cellulose-binding domain (fCBD). The gene, which we refer to as *Xyl6*, was cloned and used to generate a knockout mutant. The *Δxyl6* mutant is as virulent as its parent in the compatible rice cultivar Sariceltik. Thus, *Xyl6* is not required by *M. grisea* for pathogenicity under the infection conditions used in our experiments. To characterize the enzyme activity encoded by *Xyl6*, proteins secreted by the parent strain and the mutant were, respectively, fractionated by liquid chromatography, and each collected fraction was assayed for *endo*-β-1,4-D-glucanase and *endo*-β-1,4-D-xylanase activities. Two protein peaks with *endo*-β-1,4-D-xylanase activity secreted by the parent strain were not present in the column eluant of the proteins secreted by the mutant. The two *endo*xylanases (Xyl6-α and Xyl6-β) from the parent were each purified to homogeneity. N-terminal amino acid sequencing demonstrated that Xyl6-α is a partially depolymerized product of Xyl6-β and that Xyl6-β is identical to the protein sequence deduced from the *Xyl6* gene. Further evidence that *Xyl6* encodes an *endo*xylanase is provided by our demonstration that *Pichia pastoris* cells, transformed with the *Xyl6* gene, secrete *endo*-β-1,4-D-xylanase activity.

Wu, S.-C., A.G. Darvill, and P. Albersheim. 2003. Three differentially expressed *endo*-β-1,4-D-xylanases are required for pathogenicity in *Magnaporthe grisea*. In preparation.

Phytopathogenic fungi that infect Gramineae tend to secrete copious amount of arabinoxylan-degrading enzymes. These enzymes are proposed to be pathogenic factors for they help in breaking down the cell wall barrier, releasing oligosaccharide signal molecules and obtaining nutrients so that the fungi can enter and flourish inside plants. *Magnaporthe grisea*, the rice blast fungus, produces multiple isoforms of *endo*-β-1,4-D-xylanases under various growing conditions. We have cloned a total of six xylanase genes (*Xyl1*, *Xyl2*, *Xyl3*, *Xyl4*, *Xyl5* and *Xyl6*) that are differentially expressed both in fungal culture and in infected rice seedlings. For example, *Xyl3* transcripts were detected at very early stage (24h post-inoculation) during infection, and *Xyl4* and *Xyl5* are exclusively expressed in infected host. Infection assays of knockout mutants showed significant reduction in pathogenicity of the mutants that lack any of *Xyl3*, *Xyl4* or *Xyl5* genes. Thus, Xyl3, Xyl4 and Xyl5 appear to be pathogenicity factors of *M. grisea*. In addition, quantitative analysis of infection assays of a previously described Δ*xyl2* mutant revealed an increase in virulence. Thus, it is possible that either Xyl2 or the hydrolytic products of Xyl2 is recognized by rice as elicitor.

Wu, S.-C., Z. Zhao, A.G. Darvill, and P. Albersheim. 2003. Growth-retarded mutants of the rice blast fungus: Cloning and functional analysis of a cross-pathway control protein. In preparation.

Growth-retarded mutants (GRM) were screened from *Magnaporthe grisea* protoplasts mutagenized by random plasmid insertions. The grm phenotype of one mutant, GRM8, co-segregates with a selection marker on the plasmid. The tagged gene, *Grm8*, is structurally similar to those encoding cross-pathway control proteins (CPC) from other fungi. *Grm8* complements *cpc-1* mutant of *Neurospora crassa*, and thus is a functional ortholog of *Cpc-1*. The expression of *Grm8* is up-regulated during amino acid starvation, so are genes encoding, respectively, aspartate aminotransferase (*Aat1*) and carbamoyl phosphate synthase (*ARG2*). In strain C706, a Δ*grm8* mutant, expression of *Aat1* and *ARG2* is no longer enhanced by amino acid starvation. In amino acid-deficient media, C706 expectedly grows poorly as compared to its wild-type parent. However, in amino acid-rich media, C706 unexpectedly grows at a much slower rate than its parent before accelerating after 5 days, an indication that *Grm8* plays additional roles regulating *M. grisea* growth. In consistence with the phenotype in culture media, Δ*grm8* mutants exhibit significant reduction in pathogenicity towards rice host.

York, W.S., M. Hoffman, Z. Jia, M. Pena, M. Cash, and A. Blackburn. 2003. Structural diversity in the xyloglucans from higher plants in the subclass Asterideae. In preparation.

Xyloglucans are major components of the walls of growing and developing cell in higher plants. These polysaccharides bind spontaneously and avidly bind to the surface of cellulose microfibrils, forming a network in the cell wall that prevents the cell from bursting under osmotic stress. The controlled, orientated expansion of this network regulates the morphological changes that give mature cells their characteristic shape and size, ultimately affecting the morphology of whole tissues, organs and plants. Xyloglucan sidechains terminated in the H-antigen (α-L-Fuc*p*-(1→2)-β-D-Gal*p*) are found in xyloglucans from the cell walls of most vascular plant species, including gymnosperms, angiosperms, and even lower plants, such as ferns. Evolutionary conservation of this structural feature suggests that it is a key factor that allows xyloglucan to perform its biological function in the cell wall. However, some groups of plants, notably those from the family Solanaceae (in the subclass Asteridae), lack this structural feature, replacing it with an α-L-Ara*f* residue. Furthermore, mutation of removal of the fucosyl-transferase gene responsible for the biosynthesis of the H-antigen in xyloglucans has no obvious phenotype in *A. thaliana*, and so the selective advantage provided by this structure must not come into play under greenhouse conditions. Structural analysis of the xyloglucans from several Asterid species were analyzed to shed light on the evolutionary pattern that led to xyloglucan structural diversity in this subclass, revealing xyloglucans that contain a broad range of structural features, including xyloglucans that appear to bridge evolutionary gaps between Solanceous plants and most other plant species.

APPENDIX II
Service Activity of the DOE Center for Plant and Microbial Complex Carbohydrates

Samples Analyzed by the CCRC Plant and Microbial Carbohydrate Center
September 1, 2002 – August 31, 2003

Date of Report	Investigator	Number of Analyses	Analysis Performed
9/6/2002	Dr. Marcia Kieliszewski Department of Chemistry & Biochemistry Ohio University Athens, OH	2	linkage
9/6/2002	Mr. Tod Perry Division of Engineering & Applied Sciences Harvard University Cambridge, MA	1	composition
9/9/2002	Dr. Katie Van den Bulck Laboratorium Voor Levensmiddelenchemie Katholieke Universiteit Leuvern 3001 Heverlee, Belgium	1	linkage
9/9/2002	Dr. Nirmal Pugh National Center for the Development of Natural Products University of Mississippi University, MS	1	composition
9/11/2002	Dr. Nirmal Pugh National Center for the Development of Natural Products University of Mississippi University, MS	1	composition
9/13/2002	Dr. Maor Bar-Peled CCRC University of Georgia Athens, GA	1	composition
9/17/2002	Dr. Karin Meibom Beckman Center Stanford School of Medicine Stanford, CA	1	composition
9/20/2002	Dr. Maor Bar-Peled CCRC University of Georgia Athens, GA	16	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
9/20/2002	Dr. Mohammed Rashid Department of Epidemiology and Preventive Medicine University of Maryland School of Medicine Baltimore, MD	2	composition
9/20/2002	Dr. Paul Siitonen Division of Chemistry National Center for Toxicological Research Jefferson, AR	6	linkage
9/24/2002	Dr. Kathleen Laurenzo Danisco Terra Haute, IN	9	MALDI-MS
9/24/2002	Dr. Kathleen Laurenzo Danisco Terra Haute, IN	7	composition
10/1/2002	Dr. Lisa Friedman Department of Micro & Molecular Genetics Harvard University Boston, MA	3	composition
10/1/2002	Dr. Malcolm O'Neill CCRC The University of Georgia Athens, GA	3	composition
10/14/2002	Dr. Ginger Chew School of Public Health Columbia University New York, NY	8	composition chromotography/ protein purification
10/16/2002	Dr. Katie Van den Bulck Laboratorium Voor Levensmiddelenchemie Katholieke Universiteit Leuven 3001 Heverlee,	8	linkage
10/21/2002	Mr. James Day School of Civil & Environmental Engineering Georgia Institute of Technology Atlanta, GA	1	linkage
10/22/2002	Dr. Giuseppe Dalessandro Di.S.Te.G.A. Universita Degli Studi di Lecce 73100 Lecce Italy	8	linkage

Date of Report	Investigator	Number of Analyses	Analysis Performed
10/28/2002	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	1	MALDI-MS
10/28/2002	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	11	composition
10/29/2002	Dr. Debra Mohnen CCRC University of Georgia Athens, GA	4	composition
10/29/2002	Dr. Malcolm O'Neill CCRC University of Georgia Athens, GA	4	composition
10/30/2002	Ms. Mary O'Riordan MCB/Portnoy Laboratory University of California at Berkeley Berkeley, CA	2	composition
11/6/2002	Ms. Renee Saville Department of Civil and Environmental Engineering Stanford University Stanford, CA	1	composition
11/8/2002	Dr. Ginger Chew School of Public Health Columbia University New York, NY	5	composition molecular wgt
11/18/2002	Dr. Stephen Free Department of Biological Sciences SUNY at Buffalo Buffalo, NY	3	composition
11/20/2002	Dr. Ginger Chew School of Public Health Columbia University New York, NY	4	composition molecular wgt
11/26/2002	Dr. Ginger Chew School of Public Health Columbia University New Yrok, NY	4	composition molecular wgt

Date of Report	Investigator	Number of Analyses	Analysis Performed
12/2/2002	Dr. Matthew Parsek Department of Civil Engineering Northwestern University Evanston, IL	2	linkage
12/3/2002	Dr. Karl Kramer GMPRC-USDA-ARS Manhattan, KS	8	composition
12/3/2002	Dr. Masanori Matsukawa College of Medicine University of Iowa Iowa City, IA	2	linkage
12/3/2002	Dr. Malcolm O'Neill CCRC University of Georgia Athens, GA	2	composition
12/3/2002	Dr. Mohammed Harunur Rashid Department of Epidemiology and Preventive Medicine University of Maryland School of Medicine Baltimore, MD	2	linkage
12/9/2002	Dr. Tomo Kawaguchi School of Public Health University of South Carolina Columbia, SC	4	composition
12/12/2002	Dr. Lisa Friedman Department of Micro & Molecular Genetics Harvard University Boston, MA	2	composition
12/13/2002	Dr. Conrad Hornick Department of Physiology Louisiana State University New Orleans, LA	4	composition
12/13/2002	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	2	NMR
12/13/2002	Dr. Masanori Matsukawa Department of Microbiology University of Iowa Iowa City, IA	4	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
12/28/2002	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	6	composition
1/9/2003	Dr. Matthew Parsek Department of Civil Engineering Northwestern University Evanston, IL	1	linkage
1/13/2003	Dr. Michael Hahn CCRC University of Georgia Athens, GA	8	composition
1/14/2003	Dr. Marc Laus Institute of Molecular Plant Sciences Leiden University 2333 AL Leiden,	1	composition
1/16/2003	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	4	composition MALDI
1/21/2003	Dr. Kyle Seifert Department of Oral Biology University of Florida Gainesville, FL	7	composition
1/21/2003	Dr. Lynn Epstein Department of Plant Pathology University of California, Davis Davis, CA	2	composition linkage
1/21/2003	Dr. Lisa Friedman Department of Micro & Molecular Genetics Harvard University Boston, MA	2	composition
1/21/2003	Dr. Mary Jo Kirisits Department of Civil and Environmental Engineering Northwestern University Evanston, IL	1	composition
1/24/2003	Dr. Karin Meibom Beckman Center B237 Stanford School of Medicine Stanford, CA	1	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
2/7/2003	Dr. Lynn Epstein Department of Plant Pathology University of California, Davis Davis, CA	2	NMR
3/7/2003	Dr. Ron Orlando CCRC University of Georgia Athens, GA	1	composition
3/11/2003	Dr. Marcia Kieliszewski Department of Chemistry & Biochemistry Ohio University Athens, OH	2	linkage
3/12/2003	Erin Biers Marine Sciences University of Georgia Athens, GA	6	composition
3/13/2003	Dr. Lynn Epstein Department of Plant Pathology University of California, Davis Davis, CA	2	composition
3/17/2003	Dr. Giuseppe Dalessandro Department of Science and Technology University of Lecce 73100 Lecce Italy	6	composition
3/17/2003	Dr. Marc Laus Institute of Molecular Plant Sciences Leiden University 2333 AL Leiden The Netherlands	1	linkage
3/18/2003	Dr. Deb Mohnen CCRC University of Georgia Athens, GA	3	linkage
3/19/2003	Dr. Kathleen Laurenzo Danisco Cultor Amierca, Inc. Terre Haute, IN	10	composition linkage
3/31/2003	Mr Jeremi Johnson CCRC University of Georgia Athens, GA	3	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
4/1/2003	Dr. John Jackson Anheuser Busch St. Louis, MO	16	composition molecular wgt
4/1/2003	Dr. Kathleen Laurenzo Danisco Cultor Amierca, Inc. Terre Haute, IN	1	MALDI-MS
4/3/2003	Dr. Ginger Chew School of Public Health Columbia University New York, NY	3	composition purification
4/4/2003	Dr. John Jackson Anheuser-Busch, Inc. St. Louis, MO	18	composition molecular wgt
4/4/2003	Dr. John Jackson Anheuser Busch St. Louis, MO	19	composition molecular wgt
4/7/2003	Dr. Hassan Ahmad International Trading Pharmaceutical Laboratories, Inc. Paterson, NJ	2	composition
4/7/2003	Dr. Anthony Campagnari Department of Microbiology/Medicine University of Buffalo Buffalo, NY	5	composition
4/8/2003	Dr. Kathleen Laurenzo Danisco Cultor Amierca, Inc. Terre Haute, IN	3	composition
4/9/2003	Dr. Hassan Ahmad International Trading Pharmaceuticals Laboratories, Inc. Paterson, NJ	1	MALDI-MS
4/9/2003	Dr. Anthony Campagnari Department of Microbiology/Medicine University of Buffalo Buffalo, NY	3	composition
4/21/2003	Dr. Kathleen Laurenzo Danisco Cultor America, Inc. Terre Haute, IN	3	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
4/25/2003	Dr. David Pritchard Department of Biochemistry & Molecular Genetics The University of Alabama Birmingham Birmingham, AL	1	linkage
4/25/2003	Dr. Frank Amore Cargill Central Research Minneapolis, MN	4	composition
4/25/2003	Ms. Valerie Peters Institute of Ecology University of Georgia Athens, GA	4	composition
5/2/2003	Mr. Christian Heiss CCRC The University of Georgia Athens, GA	8	composition
5/2/2003	Dr. Marta Izydorczyk Grain Research Laboratory Canadian Grain Commission Winnipeg, Canada	1	linkage
5/6/2003	Mr. Arian Deganian CCRC The University of Georgia Athens, GA	1	composition
5/7/2003	Dr. Tllman Harder Hong Kong University of Science & Technology Clear Water Bay, Hong Kong	1	composition
5/9/2003	Dr. Kenneth Wiehe Woods Hole Oceanographic Institution Woods Hole, MA	2	composition
5/12/2003	Mr. Jonathan Cohen Duke Marine Lab Duke University Beaufort, NC	4	composition
5/15/2003	Dr. Ron Orlando CCRC The University of Georgia Athens, GA	4	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
5/19/2003	Dr. Marc Laus Institute of Molecular Plant Sciences Leiden University Leiden, The Netherlands	1	composition
5/19/2003	Ms Sajida Piperdi Albert Einstein College of Medicine Bronx, NY	1	composition
6/5/2003	Dr. Lian Li Department of Biology Pennsylvania State University University Park, PA	2	composition
6/5/2003	Dr. Paul Siitonen Division of Chemistry National Center for Toxicological Research Jefferson, AR	6	linkage
6/5/2003	Dr. Tillman Harder Hong Kong University of Science and Technology Clear Water Bay, Hong Kong	1	composition
6/6/2003	Dr. J. Luke Lentz Aluwe, LLC Minnetonka, MN	2	molecular wgt
6/7/2003	Dr. Molly Ginley Senior Analytical Chemist Aventis Pasteur Inc. Swiftwater, PA	13	1-D NMR
6/9/2003	Dr. Tillman Harder Hong Kong University of Science and Technology Clear Water Bay, Hong Kong	1	composition
6/19/2003	Dr. Michael Hahn CCRC University of Georgia Athens, GA	3	linkage
6/23/2003	Dr. Michelle Martin Wyeth Vaccines Research Pearl River, NY	14	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
7/1/2003	Dr. Christian Heiss CCRC University of Georgia Athens, GA	3	composition
7/3/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	7	composition
7/3/2003	Dr. Katie Van den Bulck Katholieke Universiteit Leuven 3001 Heverlee, Belgium	6	linkage
7/3/2003	Dr. Robert Schifferle Department of Oral Biology University of Buffalo Buffalo, NY	2	composition
7/7/2003	Dr. David Lynch Albany Medical College Albany, NY	4	composition
7/7/2003	Dr. Ginger Chew School of Public Health Columbia University New York, NY	6	protein purification molecular wgt composition
7/7/2003	Dr. Leonard Pysh Roanoke College Salem, VA	2	composition
7/9/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	1	MALDI-MS
7/9/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	21	composition
7/16/2003	Dr. Christian Raetz Medical College Duke University Durham, NC	9	composition
7/17/2003	Dr. David Domozych Department of Biology Skidmore College Saratoga Springs, NY	5	composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
7/17/2003	Dr. Pei Qian Coastal Marine Laboratory Hong Kong University Science and Technology Hong Kong	1	composition
7/22/2003	Dr. Lisa Friedman Department of Microbiology Massachusetts Institute of Technology Boston, MA	11	composition
7/24/2003	Dr. Bruce Bouché EndoMatrix, Inc. Santa Rosa, CA	2	composition
7/24/2003	Dr. John Jackson Anheuser Busch St. Louis, MO	20	molecular wgt protein purification HPLC composition
7/24/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	1	NMR
7/28/2003	Dr. W.H. Yuen Department of Chemistry University of Hong Kong Hong Kong	2	composition molecular wgt
8/4/2003	Dr. Angel Osuna School of Biological Sciences Louisiana Tech University Ruston, LA	35	composition
8/4/2003	Dr. Michelle Martin Wyeth Pharmaceuticals Inc. Sanford, NC	8	composition purification MALDI-MS
8/5/2003	Dr. Cheppail Ramachandran Miami Children's Hospital Miami, FL	1	composition
8/12/2003	Dr. Anthony Campagnari University of Buffalo Buffalo, NY	40	DOC-PAGE NMR MALDI-MS purification composition

Date of Report	Investigator	Number of Analyses	Analysis Performed
8/12/2003	Dr. Lori Hunsaker University of Utah Salt Lake City, UT	7	composition
8/12/2003	Dr. Maor Bar-Peled CCRC University of Georgia Athens, GA	12	composition
8/12/2020	Dr. Woo-Suk Chang Iowa State University Ames, IA	2	composition
8/13/2003	Dr. Giuseppe Dalessandro Dipartimento di Scienze e Technologie 73100 Lecce, Italy	6	composition
8/18/2003	Dr. Bruce Bouché EndoMatrix, Inc. Santa Rosa, CA	7	composition
8/25/2003	Dr. Cheppail Ramachandran Miami Children's Hospital Miami, FL	1	linkage
8/25/2003	Dr. Kim Sullivan SmartHealth Phoenix, AZ	12	composition molecular wgt
8/27/2003	Dr. Bruce Bouché EndoMatrix, Inc. Santa Rosa, CA	1	composition
8/27/2003	Dr. Lisa Friedman Department of Microbiology Massachusetts Institute of Technology Boston, MA	2	linkage
8/28/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	6	composition
8/28/2003	Dr. Kathleen Laurenzo Danisco Terre Haute, IN	6	composition
8/29/2003	Dr. Wenxian Sun Environmental & Plant Biology Ohio University Athens, OH	1	composition

Type of Analysis	Number of Analyses
Plant and Microbil Center:	
(9/1/02 – 8/31/03)	
Total analyses	**671**
-Glycosyl Composition	**406**
-Glycosyl Linkage	**65**
-MALDI-MS	**25**
-NMR	**23**
-Molecular weight determination	**62**
-Other	**36**

Samples and Protocols Prepared for Scientists from Industry and Other Academic Institutions

September 2002 – September 2003

Jocelyn K.C. Rose
Dept. of Plant Biology
Cornell University
Ithaca, NY 14853

1 ml CCRC-M7 antibodies

- **November 6, 2002**

Dr. Eric A. Schmelz
USDA-ARS CMAVE Chemistry
1700 SW23rd Drive
Gainesville, FL 32608

10 mg Oligogalacturonides (DP ~7-20)

- **November 11,2002**

Dr. Marcus Pauly
Plant Cell Wall Group
Max-Planck Institute for Molecular Plant Physiology
Am Mühlenberg 1
14476 Golm, Germany

3 petridishes of suspension cultured *Arabidopsis* wild-type cells

- **November 13,2002**

Dr. Tatsuya Awano
Assistant Professor
Laboratory of Tree Cell Biology
Division of Forestry and Biomaterials Science
Graduate School of Agriculture
Kyoto University
606-8502 Kyoto, JAPAN

1 ml CCRC-M1 antibodies

- **November 14, 2002**

  Dr. Paul Knox
  School of Biochemistry and Molecular biology
  University of Leeds
  Leeds LS2 9JT, United Kingdom

  2 ml CCRC-M1 antibodies

- **November 25,2002**

  Dr. Marco Moracci
  Institute of Protein Biochemistry – CNR
  Via P. Castellino 111
  80131 Naples, Italy

  900 mg Fucosylated xyloglucan

- **December 10, 2002**

  Valeska Okragly
  Lawrence University
  Biology Department
  Youngchild Hall
  115 S. Drew St.
  Appleton, WI 59411

  0.3 mg Oligogalacturonides (DP 19)
  1.4 mg Oligogalacturonides DP~ 7-20

- **January 14, 2002**

  Laurence Melton
  Food Science Programmes
  Chemistry Department
  University of Auckland
  Private Bag 92019
  Auckland, New Zealand

  3 petridishes of suspension – cultured Sycamore Cells

- **January 30, 2003**

  Prof Patrick Brown
  Dept Pomology
  U.C. Davis
  California

  25 mg Red wine RG II

- **March 14, 2003**

    Dr. Faith M. Strickland
    University of Texas
    M.D. Anderson Cancer Center
    Dept. of Immunology, Box 178
    1515 Holcombe Blvd.
    Houston, TX 77030

    2.0 mg Octamer XXLG
    3.2 mg Nonamer XXFG
    4.0 mg Heptamer XXXG
    2.9 mg Decamer XLFG
    2.7 mg S2

- **May 5,2003**

    Eliot Herman
    Molecular Biologist
    USDA and
    Danforth Plant Science Center
    Washington University in St. Louis
    St. Louis, MO

    1 ml CCRC-M1 antibodies

- **May 5, 2003**

    Philip J. Harris
    Associate Professor
    School of Biological Sciences
    The University of Auckland
    Private Bag 92019
    Auckland, New Zealand

    1 ml CCRC-M1 antibodies

- **June 9,2003**

    Dr. Olga Zabotina
    University of California
    Department of Botany & Plant Sciences
    2116 Batchelor Hall
    Riverside, CA 95521-0124

    15 mg Oligogalacturonides DP~7-20

- **June 10,2003**

  Dr. Debra Mohnen/Crystal Jackson
  Complex Carbohydrate Research Center
  The University of Georgia
  Athens, GA 30602

  15 mg Rhamnogalacturonan-I
  25 mg Rhamnogalacturonan II
  20 mg Oligogalacturonides

- **June26, 2003**

  Naomi Geshi
  Biotechnology Group
  Danish Institute of Agricultural Sciences
  Copenhagen, Denmark

  15 mg Rhamnogalacturonan II

- **June 27,2003**

  Crystal Jackson
  Complex Carbohydrate Research Center
  The University of Georgia
  Athens, GA 30602

  15 mg Rhamnogalacturonan I

APPENDIX III.

Extramural Training Courses

Three extramural training courses were offered at the CCRC during 2003.

Two of the three extramural training courses were offered at the CCRC in June 2003. Experience with basic biochemical techniques is a prerequisite for participation. Each course lasts one week. These two courses were offered back-to-back again this year because there are always several participants who wish to participate in both courses.

Course 1: Analytical Techniques for Carbohydrate Structure Determination, June 16-20; 10 participants

Course 2: Separation and Characterization of Glycoconjugate Oligosaccharides, June 23-27, 14 participants

These training courses were organized by Dr. Parastoo Azadi. Each course consisted of lectures, demonstrations by the course leaders and selected CCRC staff, and hands-on laboratory work for the trainees. In addition, each course participant could chose from one of two modules: *Mass Spectrometric Analysis of Glycoconjugates* or *NMR Spectroscopic Analysis of Glycoconjugates*. These one-day sessions were led by Dr. Ron Orlando and Dr. John Glushka, respectively. A laboratory manual, which is updated annually, was provided to each course participant and included more detailed information on selected analytical techniques as well as a bibliography.

Course 1 (*Analytical Techniques for Carbohydrate Structure Determination*) covered the techniques of glycosyl residue (composition) and glycosyl linkage (methylation) analyses using gas-liquid chromatography/mass spectrometry. Participants performed analyses of purified oligosaccharides, and several brought their own samples. Lectures and demonstrations covered other techniques for structural analysis. In addition, a choice of modules either on using mass spectrometry or NMR spectroscopy for analysis of glycoconjugates was offered (see descriptions below). Experience with basic biochemical techniques is a prerequisite for participation

Course 2 (*Separation and Characterization of Glycoconjugate Oligosaccharides*) Participants will learn basic techniques for the isolation and characterization of oligosaccharides via a combination of hands-on work, lectures and demonstrations. Mixtures of oligosaccharides or glycopeptides derived from glycoproteins will be resolved into individual components by chromatographic procedures such as size-exclusion, HPLC, lectin affinity chromatography, and/or polyacrylamide gel electrophoresis. Glycosphingolipids will be separated by thin-layer chromatography, and detection protocols will be discussed. The use of lectin blotting techniques for characterization of oligosaccharide structural features will be covered. Other topics to be included are conjugation of oligosaccharides with fluorescent tags, capillary electrophoresis and monosaccharide composition analysis. In addition, modules on using mass spectrometry and/or NMR spectroscopy for analysis of glycoconjugates will be included (see descriptions below). Experience with basic biochemical techniques is a prerequisite for participation.

These courses were announced on the CCRC's website as well as on other appropriate websites and in several University of Georgia publications. We contacted clients for whom we have done analytical service. The registration fee per course was $500 for individuals from nonprofit institutions and $1,100 for others.

Additional Course Modules

***Mass Spectrometric (MS) Analysis of Glycoconjugates*:** The use of mass spectrometry for the characterization of glycoconjugates was discussed. Topics in this area included identifying occupied glycosylation sites by LC-MS and sequencing glycoprotein glycans with sequential exoglycosidase digestions followed by MS. Laboratory demonstrations were performed on these two topics.

***NMR of Carbohydrates*:** This module was a basic introduction to the application of NMR spectroscopy of polysaccharides and glycoconjugates covering *i)* essential features of NMR spectra, *ii*) protocols used to solve primary structures, *iii*) applications and examples from the current literature, and *iv*) resources for information. Our four NMR instruments (300-, 500-, 600- and 800 MHz) were used for demonstrations during this module.

Course 3: Mass Spectrometry of Glycoproteins, April 14-15;14 participants

In response to the large number of requests that we had received regarding offering a more in depth course on mass spectrometry glycans, Dr. Azadi organized and offered a 2-day (April 14 &15) course on mass spectrometry of glycoproteins. Lectures taught by both Drs. Azadi and Orlando included mapping the glycosylation sites in glycoproteins; determining the composition, sequencing, and branching points of N and O-linked oligosaccharides, and MS procedures used in these analyses. Demonstrations were performed on both matrix-assisted laser desorption (MALDI) and electrospray ionization (ESIMS) instruments. A course manual including selected analytical techniques and references was provided to each course participant. The cost of registration was $200 per course for individuals from non-profit institutions and $400 for others. There were 14 participants, 9 from non-profits institutions and 5 from industry.

Participants

Course 1

Linda Goding
Department of Urology
Medical College of Ohio
Toledo, OH

Gerald Hofmann
Biocenbtrum-Dtu
Lyngby, Denmark

Kathryn Jones
Massachusetts Institute of Technology
Cambridge, MA

Gabre Kemp
CCRC
University of Georgia
Athens, GA

Hyunwoo Lee
Department of Molecular Microbiology
Washington University
School of Medicine
St. Louis, MO

Rebecca Lombard
Centocor
Malvern, PA

Sara Petersson
Danish Institute for Agricultural Science
Frederiksberg, Denmark

Mike Segura
Halliburton Energy Services
Duncan, OK

Elizabeth Sutton
Amgen
Seattle, WA

Hui Wu
Department of Molecular Genetics
and Microbiology
University of Vermont
Burlington, VT

Participants

Course 2

Liz Adam
University of Southampton
Southampton, UK

Peggy Angel
CCRC
University of Georgia
Athens, GA

Jonathan Cohen
Duke University
Beaufort, NC

Brett Forshey
Vanderbilt University
Nashville, TN

Naomi Geshi
Danish Institute of Agricultural Sciences
Frederiksberg, DK

Linda Goding
Department of Urology
Medical College of Ohio
Toledo, OH

Lorraine Hill
Aventis Pasteur
Swiftwater, PA

Kathryn Jones
Massachusetts Institute of Technology
Cambridge, MA

Gabre Kemp
CCRC
University of Georgia
Athens, GA

Hyunwoo Lee
Department of Molecular Microbiology
Washington University School of Medicine
St. Louis, MO

Darren Locke
University of Maryland New Jersey
Newark, NJ

Rebecca Lombard
Centocor
Malvern, PA

Amy Martin
Centers for Disease Control
Atlanta, GA

Malin Moisander
Biovitrum
Stockholm, Sweden

Participants

Course 3

Gerald Alvarez
Department of Biochemistry
And Molecular Biology
University of Georgia
Athens, GA

Laura Barrientos
National Institutes of Health
Bethesda, MD

Letha Chemmalil
Altus Biologics
Cambridge, MA

Bill Dollard
Acorda Therapeutics, Inc.
Hawthorne, NY

Huaiyu Hu
MetroHealth Medical Center
Cleveland, OH

Maria Kamar
Department of Biochemistry
and Molecular Biology
University of Georgia
Athens, GA

Gabre Kemp
CCRC
University of Georgia
Athens, GA

Paul Kodama
Amgen
Seattle, WA

Jin Kyu Lee
Department of Biochemistry
and Molecular Biology
University of Georgia
Athens, GA