Techniques for Characterization of Carbohydrate Structure of Plant and Microbial Polysaccharides

August 5-9, 2019
Room 1036 (first floor of building)

LABORATORY EXERCISE

Section I
Alditol acetate (AA) composition

Section II
Methyl glycosides (TMS) composition

Section III
Partially methylated alditol acetate (PMAA) linkage

Lab Instructors: Zhirui Wang, Sara Porfirio, and Ian Black
Monday, August 5, 2018

9:00 a.m. – 9:15 a.m.
Welcome and Introduction
Dr. Parastoo Azadi

9:15 a.m. – 9:30 a.m.
Hydrolysis of AA sample

9:30 a.m. – 10:15 a.m.
**Lecture** – “Structural Characterization of Carbohydrates” (Part 1)
Dr. Parastoo Azadi

10:15 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 11:00 a.m.
**Lecture** – “Structural Characterization of Carbohydrates” (Part 2)
Dr. Parastoo Azadi

11:00 a.m. – 12:15 p.m.
Remove AA sample from heat and dry down with nitrogen
Sodium borodeuteride reduction of AA sample

12:15 p.m. – 1:15 p.m. – **LUNCH**

1:15 p.m. – 2:15 p.m.
Neutralization and drying of AA sample
Acetylation of AA sample

2:15 p.m. – 3:45 p.m.
**Lecture** – “Different Forms of D-Glucose”
Dr. Geert-Jan Boons

3:45 p.m. – 4:00 p.m. – **Break**

4:00 p.m. – 5:00 p.m.
Drying, extraction, and injection of AA sample
Overnight methanolysis of methyl glycosides (TMS) sample
**Tuesday, August 6, 2018**

9:00 a.m. – 9:30 a.m.
- Dry down methyl glycosides (TMS) sample
- Prepare NaOH base for permethylation (Linkage)

9:30 a.m. – 10:25 a.m.
- Permethylation of the linkage sample
- N-acetylation of methyl glycosides samples (TMS)

10:25 a.m. – 10:40 a.m. – Break

10:40 a.m. – 11:15 a.m.
- Remove the CH₃I, DCM Extraction and drying (Linkage)
- Drying of the N-acetylated methyl glycosides (TMS) sample after 30 min reaction

11:15 a.m. – 12:15 p.m.
- Lecture – “Isolation and Characterization of Bacterial Surface Polysaccharides”
  Dr. Artur Muszynski

12:15 p.m. – 1:15 p.m. – LUNCH

1:15 p.m. – 2:30 p.m.
- Silylation of the methyl glycosides (TMS) sample

**Laboratory Demonstration (Lab 1025)**
- Preparation of (dmsyl) potassium dimethylsphynyl anion

2:30 p.m. – 3:45 p.m.
- Lecture – “A developing hypothesis: GAUTs synthesize pectic domains required for plant cell wall architecture: An example of how “polysaccharide” structural determination provides unique understanding”
  Dr. Debra Mohnen
3:45 p.m. – 4:00 p.m. – Break

4:00 p.m. – 4:15 p.m.

Lecture – “FACES Scheduling”
Saeid Roushanzamir

4:15 p.m. – 5:00 p.m.

Laboratory
Drying, centrifugation, and injection of the methyl glycosides (TMS) sample

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**Wednesday, August 7, 2018**

9:00 a.m. – 9:15 a.m.
Hydrolysis of PMAA sample

9:15 a.m. – 9:45 a.m.

Demystification – Lignin Analysis by Pyrolysis - Molecular Beam - MS
John Tang

9:45 a.m. – 10:00 a.m. – Break

10:00 a.m. – 11:00 a.m.

Lecture – “Glycome Profiling“
Dr. Michael Hahn

11:00 a.m. – 12:15 p.m.
Remove linkage sample from heat and dry down with nitrogen
Sodium borodeuteride reduction of linkage sample

12:15 p.m. – 1:15 p.m. – LUNCH

1:15 p.m. – 2:30 p.m.

Neutralization and drying of PMAA sample
Acetylation of PMAA sample

2:30 p.m. – 3:15 p.m.
Data analysis of AA
3:15 p.m. – 3:30 p.m. – **Break**

3:30 p.m. – 4:15 p.m.

Lecture – “Analytical tools for structural characterization of peptidoglycan”

**Dr. Sara Porfirio**

4:15 p.m. – 5:00 p.m.

Drying, extraction, and injection of PMAA sample

**Thursday, August 8, 2018**

9:00 a.m. – 10:00 a.m.

Data analysis of TMS

**Dr. Zhirui Wang**

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 11:15 a.m.

**Lecture** – “Labeling and Separation of Carbohydrates”

**Dr. Stephanie Archer-Hartmann**

11:15 a.m. – 12:15 p.m.

Data analysis for linkage

**Ian Black**

12:15 p.m. – 1:15 p.m. – **LUNCH**

1:15 p.m. – 1:35 p.m.

HPAEC demonstration: Oligosaccharide profiling analysis and monosaccharide composition analysis

**Dr. Zhirui Wang**

1:35 p.m. – 2:45 p.m.

Questions and answers for data interpretation of all the analyses

**Drs. Wang and Azadi**

2:45 p.m. – 3:00 p.m. – **Break**
3:00 p.m. – 4:00 p.m.

Lecture – “Introduction to Xyloglucan Structure and Biosynthesis”
Dr. Breeanna Urbanowicz

4:00 p.m. – 5:00 p.m.

Lecture “Introduction to High Performance Anion Exchange Chromatography”
Dr. Parastoo Azadi

Friday, August 9, 2018

<NMR Module>

9:00 a.m. – 12:00 p.m.

Lecture – “Introduction to NMR of Glycoproteins and Carbohydrates”
Dr. John Glushka

12:00 p.m. – 1:00 p.m. – LUNCH

Afternoon:

Demonstration and data interpretation

Or <Mass Spectrometry Module>

9:00 a.m. – 12:00 p.m.

Lecture – “Mass Spectrometry analysis of oligosaccharides”
Dr. Parastoo Azadi

Afternoon:

Demonstration and data interpretation

Or <Molecular Modeling Module>

9:00 a.m. – 12:00 p.m.

Lecture and Demo – “Introduction to Molecular Modeling”
Dr. Lachele Foley

12:00 p.m. – 1:00 p.m. – LUNCH
Afternoon:

Demonstration

Course summary, Course evaluation, Final Questions and Answers