Plant Microbiology Lab
SYLLABUS 2014

Course Overview: The focus will be on measuring the impacts of beneficial microorganisms on plants. Assays will include colonization of the plants, N stress alleviation, photosynthesis, and overall growth enhancement. In addition, microorganisms will be characterized in terms of phytohormone production, provision of nutrients, and growth, as well as a molecular analysis of the strains. Each class period will begin with a short lecture on the experiments for that period.

Learning Goals/Objectives: The students will learn a variety of valuable laboratory skills and gain an appreciation for the importance of plant microbe symbioses. Basic microbiology skills including making media, pouring plates, streaking strains for single colony isolation, inoculating broth, and measuring optical density will be taught. Phytohormone production will be quantified as an example of a biochemical assay. Standard microscopy techniques will be learned. Basic molecular biology skills including colony PCR, electrophoresis, DNA purification, ligation, transformation, plasmid purification, and sequence analysis will be taught. A variety of plant physiology tests will be performed such that the students will learn the full range of plant microbiology skills.

Required Readings: The students will be required to read reviews and specific scientific papers relating to the labs. There will be no required text book.

Evaluation and Grading: Participation in each lab class period is required. Grading will be based on three written lab write-ups after completion of each major experiment (25% each for 75% of total grade). Detailed lab notebooks outlining the experiment, purpose of the lab, materials and methods, and results is required along with pre-lab assignments. The lab notebooks with pre-lab assignments will be assessed throughout the quarter (25% of grade).

Weekly Course Schedule (Overview):

Mon Jan 6	Introduction to the lab; discussion of lab safety. Prof. Doty is at a required meeting so this intro will be led by the two TA's: Tony Rho and Shyam Kandel
Fri Jan 10	Preparing media; pouring plates; starting lab notebooks; streaking strains for single colony isolation. Begin phosphate solubilization assay. (Doty away)
Mon Jan 13	Discussion of the strains being used in the lab. Check plates for single colonies & contamination. Streak strains on NF-CCM and MGL. Start cultures for quantification of phytohormone production.
Fri Jan 17	Assay for hormone (IAA) production by microbes. Quantify phosphate solubilization by measuring the clearing zone (if any); compare to controls.
Mon Jan 20	Holiday
Fri Jan 24	Inoculate flasks of NF-CCM and MGL. Prepare pots with soil and labels for next week. Saturate soils fully with water. Siderophore production assay.
Mon Jan 27	Measure OD_{600} of a dilution of the cell suspension; calculate amounts needed for inoculum; make dilutions in Qubit NFM, verify OD, inoculate seeds in soil; ctrl seeds get only medium (no microbes) in Qubit NFM. Results of siderophore assay

Fri Jan 31 Discussion on strain identification methods. Colony PCR for 16S rDNA gene
Lab write-up #1 on the IAA Production Experiment and Phosphate Solubilization is due.

Mon Feb 3 Meet at CUH greenhouse. Measure seedlings (1 wk data). Remove extra seedlings that did not germinate well.
Fri Feb 7 Pour agarose gels; electrophoresis; cut out 16S bands; store at 4C.

Mon Feb 10 Meet at CUH greenhouse. Measure seedlings (2 wk data)
Fri Feb 14 Elute DNA for cloning; ligate to cloning vector

Mon Feb 17 Holiday
Tuesday In your own time, go to CUH and measure seedlings (3 wk data)
Fri Feb 21 Transformation of E. coli with the ligations

Mon Feb 24 Meet at CUH greenhouse. Measure seedlings (4 wk data); Photosynthesis measurements.
Fri Feb 28 Plasmid DNA preparations for sequencing

Mon Mar 3 Measure seedlings (5 wk data). Other plant phys expts?
Early in the week: your sequencing results will be emailed to you
Fri Mar 7 *Lab write-up #2 on Strain Identification (molecular biology experiments) and siderophore assay is due.*

Mon Mar 10 End the seedling experiments. Photographs after harvest; measure lengths of shoots; cut roots from shoots and weigh separately in labeled aluminum dishes (wet weights).

Fri Mar 14 Weigh dried tissues; add data to spreadsheets; class data analysis. Course evaluations; turn in lab notebooks for grading

Final Exam Day: Written report on Effects of Endophytes on Corn Growth and Physiology, including an analysis of the overall class data of the seedling experiments