

ESRM 404: Plant Microbiology Lab
Summer Quarter (June 18-Aug 17, 2018)
5 credits; MWF 940-1120 WFS 107

Course Overview: The course will introduce the broad topic of plant microbiology and then focus on measuring the impacts of beneficial microorganisms on plants. Assays will include colonization of the plants, salt 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. Phosphate solubilization and siderophore production will also be assayed as they are common characteristics of plant-associated microorganisms. Standard microscopy techniques will be learned. Basic molecular biology skills including colony PCR, electrophoresis, 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. These will be provided on our Canvas website. There will be no required text book.

Evaluation and Grading: Participation in each lab class period is required. Grading will be based on written lab reports (25% mid-term and 50% final report). These should outline the experiments, purpose of the lab, materials and methods, results, and discussion. Pre-lab assignments will be assessed throughout the quarter (25% of grade).

Draft Syllabus:

Mon June 18: Lecture: Intro to the lab and the project; discussion of lab safety; starting lab notebooks; symbiosis (mutualists and pathogens); overview of microbial assays for potentially symbiotic traits; start flats of tomato seeds and beans (could do some beans extra as a N-fixation demo +/- Rhizobium)

Wed June 20: Lecture: Microbiological assays for strain characterizations

Lab: Sterile technique; pouring plates (rich medium, N-limited medium, insoluble phosphate plates, siderophore assay plates); view microbial diversity on plates and through microscope (descriptions)

Fri June 22: Lecture: Microbial conjugations and fluorescence microscopy

Lab: Streaking strains for single colony isolation on MG/L plates; streaking on selective media for N and P and antibiotic sensitivities. View GFP and RFP donors

Mon June 25: Lecture: Inoculation of plants; review of assays started last week

Lab: Check plates for single colonies & contamination. Check for phosphate solubilization activity. Check results of antibiotic resistance plates. Start cultures for conjugations and inoculations. Prepare pots with soil and labels for next week. Saturate soils fully with water or salt water

Wed June 27: Lecture: Using a spectrophotometer; why we need it

Lab: Measure optical density of cultures; adjusting OD; preparing inoculum; inoculating seedlings. Set up conjugations to introduce fluorescent tags

Fri June 29: Lecture: Discuss results so far

Lab: Plate conjugations onto selective medium

Prof. Doty is away July 1-July 10 and July 16-July 20

Mon July 2: Intro to plant assays; transplant inoculated and control seedlings to the regular or salty soil; time zero height measurements

Wed. July 4: HOLIDAY

Fri July 6: Measure stomatal conductance and stomata imprints assay; Chlorophyll content (SPAD)

Mon July 9: Height measurements (1 wk); Observe the stomata imprints samples (frequency of open vs closed)

Wed July 11: Photosynthetic activities (Fv/Fm)

Fri July 13: Chlorophyll content (SPAD)

Mon July 16: Height measurements (2 wk)

Wed July 18: Photosynthetic activities (Fv/Fm)

Fri July 20: Measure stomatal conductance and stomata imprints assay; Chlorophyll content (SPAD)

Mon July 23: Discussion on strain identification using molecular biology techniques; Fluorescence plasmid results; Height measurements (3 wk); Observe the stomata imprints samples

Wed July 25: Colony PCR for 16S rDNA gene

Fri July 27: Electrophoresis; Begin anti-fungal activity assays

Mon July 30: Sample prep for sequencing; Height measurements (4 wk)

Wed Aug 1: Sequence analysis to identify the strains

Fri Aug 3: Results of anti-fungal assays

Mon Aug 6: Height measurements (5 wk); stress assays (ROS)

Wed Aug 8: Harvest: wash roots, photos; cut roots from shoots and weigh separately in labeled aluminum dishes (wet weights). For the shoots, we are using paper bags since they are bigger.

Fri Aug 10: Weigh dried tissues; add data to spreadsheets;

Mon Aug 13: Class data analysis.

Wed Aug 15: Lecture on stats

Fri Aug 17: Last day of class. Compile all the data and discuss