ESRM 404: Plant Microbiology Lab Summer Quarter (Full) 2017

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, 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. 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 three written lab write-ups after completion of each major experiment (25% each for 75% of total grade). Submission will be through the Canvas website. 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:

Week	TOPICS
1 (6/19) M	**Lecture**: Introduction to the lab and the project; discussion of lab safety; starting lab notebooks; symbiosis (mutualists and pathogens) **Lab**: View microbial diversity on plates and through microscope; pour plates (rich medium, N-limited medium, and insoluble phosphate plates); streaking strains for single colony isolation on MG/L plates.
1 (6/23) F	**Lecture**: Microbiological assays for strain characterizations; microbial conjugations (for next week). **Lab:** Check plates for single colonies & contamination. Begin phosphate solubilization assay. Antibiotic resistance assay. Antifungal assay. Streak strains for single colony isolation (perfect the technique). Start cultures for optical density measurements and for conjugations
2 (6/26) M	**Lab**: Results of antibiotic resistance assay and P solubilization assay. Streak strains on N-

	limited medium and rich medium. Start cultures for quantification of phytohormone production. Conjugations with fluorescent marker strains
2 (6/30) F	**Lecture/Lab**: Assay for hormone (IAA) production by microbes. Plate conjugations on selective media.
3 (7/3) M	**Lecture**: Intro to plant assays (prep for inoculation). **Lab:** Streak-purify fluorescently-labeled strains on medium with antibiotics. Inoculate flasks of NL-CCM and MG/L. Prepare pots with soil and labels for Friday. Saturate soils fully with water; plant seeds. Siderophore production assay.
3 (7/7) F	**Lecture/Lab**: Inoculation. 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. (TAs will move the pots to the CUH greenhouse over the weekend)
4 (7/10) M	**Lab:** Discussion on strain identification using molecular biology techniques. Colony PCR for 16S rDNA gene.
4 (7/14) F	**Lecture/Lab:** Meet at CUH greenhouse. Introduction to plant physiology assays. Greenhouse tour and safety training. Measure seedlings (wk 1 data). Remove extra seedlings that did not germinate well. ** Lab write-up #1 on the microbial characterization (IAA production experiment, P solubilization; siderophore production; general growth features; morphology; fungal assay results) is due (25% of total grade).*
5 (7/17) M	**Lab**: Electrophoresis; sample prep for sequencing; nifH analysis. Fluorescence plasmid results. Check fluorescently-tagged strains using microscope.
5 (7/21) F	**Lecture/Lab**: Meet at CUH greenhouse. Photosynthetic activities. Measure seedlings (wk 2 data), chlorophyll content (SPAD) and photosynthetic activities (Fv/Fm)
6 (7/24) M	**Lab**: Chemotaxis assay. Quantify your PCR products in preparation for sequencing.
6 (7/28) F	**Lecture/Lab**: Meet at CUH greenhouse. Stomatal functions. Measure stomatal conductance and stomata imprints assay. Measure seedlings (wk 3 data)
7 (7/31) M	**Lab:** Sequence analysis to identify the strains. Compile class data. Chemotaxis assay results, Observe the stomata imprints samples
7 (8/4) F	**Lab:** Meet at CUH greenhouse. Measure seedlings (wk 4 data). Give brief instructions on harvesting and measuring biomass process for next week. ** Lab write-up #2 on strain identification (molecular biology experiments) and further characterizations (chemotaxis; fluorescence microscopy) (25% of total grade).*
8 (8/7) M	**Lab:** Meet at CUH greenhouse. End the seedling experiments. Photographs after harvest; measure lengths of shoots; cut roots from shoots and weigh separately in paper bags.
8 (8/11) F	**Lab**: Meet at CUH greenhouse. Weigh dried tissues; add data to class spreadsheets;
9 (8/14) M	**Lecture/Lab**: Data analysis using MS Excel (workshop). ** Turn in lab notebooks for final grading. (25% of total grade)*
9 (8/18) F	**Lecture/Discussion**: Class data analysis/discussion (prepared by TAs/instructor). Paper discussions about plant microbial symbiosis. (discussion papers TBD). ** Lab write-up #3: on effects of endophytes on plant growth and physiology, including an analysis of the overall class data of the seedling experiments (25% of total grade)*