

Plant Microbiology Lab- ESRM490B: M&F 9:30-11:20 AM
SYLLABUS- Revised 2/1/13

The focus will be on the impacts of endophytes on corn and rice: colonization, growth in low nutrients, N stress alleviation, water use efficiency, and drought tolerance. Creation of this lab class was funded by a grant from the USDA/NIFA program on Climate Change Mitigation

- Mon Jan 7 Introduction; lab safety
Fri Jan 11 Melt agar; pour plates; start lab notebooks; streak strains
- Mon Jan 14 Check plates for single colonies & contamination. Streak strains on NF-CCM; start cultures for hormone quantification
Fri Jan 18 Measure hormone production by microbes; Measure colony sizes on NF-CCM
- Mon Jan 21 Holiday
(Tues Jan 22: TAs will streak strains on MGL)
Fri Jan 25 Inoculate flasks of NF-CCM and MGL. Prepare pots with soil and labels for next week (#2 soil for N fix expts; #4 soil for drought expts). Weigh cells for IAA experiment. Calculate IAA produced per mass of cells. Add to lab chart for characterization of the strains.
- Mon Jan 28 Measure OD₆₀₀ of a dilution of the cell suspension; calculate amounts needed for inoculum; make dilutions, verify OD, inoculate seeds; ctrl seeds get only medium (no microbes)
(Tues or Wed- TAs replace inoculum with sterile water)
Fri Feb 1 Transfer controls and inoculated seedlings to labeled pots of 2 soil types; introduction to fluorescent microscopy
- Mon Feb 4 Check colonization by GFP and RFP strains; streak un-ID strains onto NF-CCM
Fri Feb 8 Measure seedlings (1 wk data); colony PCR for 16S rDNA gene
- Mon Feb 11 Pour agarose gels; run gels; cut out 16S bands; store at 4C
Fri Feb 15 Measure seedlings (2 wk data); elute DNA for sequencing
- Mon Feb 18 Holiday
Fri Feb 22 Measure seedlings (3 wk data); analyze and discuss sequencing data. Repeat?
- Mon Feb 25 Start drought test (seedlings in #4 soil)
Fri Mar 1 Measure seedlings (4 wk data); fungal observations
- Mon Mar 4 Observe/record drought stress data (one week); fungal lab led by Nick Herscherberger
Fri Mar 8 Measure seedlings (5 wk data); Leaf color charts for N stress
- Mon Mar 11 Plant physiology measurements? WUE? Stomatal differences?
Fri Mar 15 Measure seedlings (6 wk data); final tests for effects of endophytes
Final Exam Clean up. Turn in lab notebooks for grading