BIMM 121 Laboratory in Microbiology
Winter 2012

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Office hours: Tuesdays 11 to 12 noon
Lecture: TuTh - 12:30p - 1:50p in SEQUO 147
Labs: TuTh - 2:30p - 6:30p in York Hall 2310 and 2332

Teaching Assistants
Portia Sachi Lombardo
Deron Trent Amador

Course Structure:

This course will introduce you to the fundamentals of microbiology and allow you to explore the many ways in which microbes affect and are used in our lives. We begin the course with a foundation in basic techniques such as sterile techniques, microscopy, methods of quantitating microbes, and preparing and examining stained slides. The remaining duration of the course will comprise four main units: a comprehensive look at bacterial physiology, understanding the complex microbial community of soil, metagenomics as a tool in exploring complex communities, and the use of microbes in various aspects of our lives. Each of these units comprises several multi-day experiments and there will be considerable overlap in the execution, methodology, and analysis of data from each of these units. Throughout the course, you will also receive training in accurate data entry and analysis, scientific reasoning, and in clear and concise scientific writing.

Equipment:

For this lab you will need to purchase:

- A lab notebook WITH carbon copies.
- A lab coat; and,
- Eye protection (you may wear either safety glasses or goggles, but standard prescription eye glasses are not sufficient).

Attendance and Absences:

- Your attendance is required at EVERY lab and through the entire lab period, until all the experimental work for the day is completed.
- Absences will NOT be treated lightly. The labs are set up for groups of two or more and your absence will place an unnecessary burden on your partner. There are no make up labs and you will not be allowed in the lab on non-lab days or in the other Micro lab sections, although you may be asked to make up the work from the day you missed.

- Documentation will be required for all unavoidable absences.
- If you are likely to have interviews for graduate school, etc., please schedule them on non-lab days.
- All absences without prior notification/permission and the appropriate paperwork will be considered unauthorized.
- 50-point penalty for the first unauthorized, unexplained absence from the lab. If there is a second such absence, you will be asked to drop the course.
- If you are ill on a lab day or have an emergency, e-mail or call (instructor or lab partner) before the start of the lab. If you are ill enough to miss lab you must go to the student health center and provide documentation of your illness.

Assignment Deadlines and Submission:

1. A hard copy of each lab report is due in the first 20 minutes of the lecture period of the day on which your report is due. Reports turned in more than 20 minutes after the start of class will be considered late. Penalty for late reports will be 10% for each day late. Lab reports submitted after 3 days will not be accepted for grading.
2. In addition to the hard copy of the report, you are required to submit an electronic copy to Turnitin.com. A link to the e-submission website will be provided on ted. Failure to submit on Turnitin.com will results in 0 (zero points) recorded for that report. Check the deadline of the Turnitin.com submission and make sure you adhere to it. Students agree that by taking this course all required papers would be subject to review for textual similarity by Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin.com service is subject to the terms of use agreement posted on the Turnitin.com site.
3. Additional points may be taken for late electronic submissions.
4. Homework assignments when applicable must be turned in as hard copies (exceptions being the online survey and online quizzes). They are due within 10 minutes of the start of the lecture. Late submissions will be cost points (10% per day).

Regrade Requests:
All regrade requests should be submitted in writing within one week of receiving the graded material.

Grading scheme:

Note Book (4 random checks at 10 points each)	40
Pop quizzes (6 pop quizzes at 5 points each)	30
Lab performance and participation	30
Home works (8)	100
Lab report - 1	25
Lab Report - 2	100
Lab Report -3	100
Midterms I	50
Midterm II	50
Midterm III	75
Total points	**600**

Final grade:
The final grade is based on a straight average of your scores.
97+ = A+
93–96 = A
90-92 = A-
87-89 = B+
83-86 = B
80-82 = B-
76-79 = C+
70-75 = C
68-70= C-
60-68 = D
Less than 60 = F

How to use your notebook

Table of contents
Start a new page each day … and for each experiment.
Do not leave pages blank to fill in later. You **may not** record data retroactively.
For each experiment, include:
Purpose of experiment
Procedure
- Outline, or page from which protocol was taken
- Note any changes
- Note who did which part of the procedure
- – who inoculated controls, etc
- Note which organisms you used
- – name and species of the controls, etc
- Record any errors

Observations
Write
Draw
Questions and connections
Conclusion or summary
Answer any questions in the manual or that were raised in class.

Lab Performance and Participation
In addition to quizzes, midterms, lab reports and assignments, student evaluations will be based on the following criteria:
1. Lab techniques will be evaluated in class
2. Lab workshop participation
Subjective student evaluations will be based on the following criteria:
3. Pre-lab preparation
4. Careful management of lab procedures (e.g., sterile technique, proper waste disposal, experimental procedures, etc.)
5. Ability to adapt to unforeseen procedural changes
6. Caliber of thinking before asking questions
7. Scientific approach (e.g., proper use of notebooks, controls, experimental design)

8. Accuracy
9. Independence
10. Safety consciousness
11. General neatness in lab

Please note: You will be expected to get into the habit of methodical, well-planned and organized work by the mid-term. This will help you with the experiments in the second half of the course.

Course Website
This course is on WebCT (https://webctweb.ucsd.edu) and should automatically appear on your WebCT account as soon as you register for the class. We will use WebCT to post information on experiments, exams, schedules, readings and practice material, experimental data, report guidelines, etc. We strongly encourage you to use the Discussion board to post questions or answers to questions and to use it as a forum for exploring the material. The TAs and I will routinely check this website and answer any questions but feel free to respond as well. This website will also be used to post any announcements that pertain to the entire class. Please check the site regularly and update yourself on the information provided.

University Policy on Integrity of Scholarship
The principle of honesty must be upheld if the integrity of scholarship is to be maintained by an academic community. The University expects that both faculty and students will honor his principle and in so doing protect the validity of University grading. This means that all academic work will be done by the student to whom it is assigned, without unauthorized aid of any kind. Instructors, for their part, will exercise care in planning and supervising academic work, so that honest effort will be encouraged.

Student Responsibility:
Students are expected to complete the course in compliance with the instructor's standards. No student shall engage in any activity that involves attempting to receive a grade by means other than honest effort; for example:

- No student shall knowingly procure, provide, or accept any unauthorized material that contains questions or answers to any examination or assignment to be given at a subsequent time.
- No student shall complete, in part or in total, any examination, or assignment for another person.
- No student shall knowingly allow any examination or assignment to be completed, in part or in total, for himself or herself by another person.
- No student shall plagiarize or copy the work of another person and submit it as his or her own work.
- If any work is plagiarized from that of another student, both students will be reported to the Office of Academic Integrity, even if one of the students has graduated already. Remember that most graduate schools check the undergraduate records for any indications of dishonesty before awarding a degree.
- No student shall alter graded class assignments or examinations and then resubmit them for regrading.
- No student shall submit substantially the same material in more than one course without prior authorization.

Lab	Date	Experiment	Reports, Midterms, Reminders
Lab 1	Tues/Wed April 3/4	1. Registration, attendance, safety video, responsibility agreements, introductory remarks, 2. Safety lecture **Sterile technique.** 3. Microbes in the environment 4. Why wash your hands? **Use of pipettors:** Demo and exercise **Plant pathogen interaction:** Inoculate *Kalanchoe* plant with *Agrobacterium*	
Lab 2	Thurs/Fri April 5/6	**Sterile technique.** 1. Microbes in the environment: Observe results 2. *E. coli* and toilet paper experiment: Observe results 3. Aseptic technique: streak and spread plates 4. Demo 5. Lab exercise using a mixed bacterial culture **Microscopy:** 1. Learning to focus the light microscope 2. Demo 3. Lab exercise using prepared (commercial) slides 4. Cleaning your microscope – demo and completion	**HW 1 due: pre-workshop library survey**
Lab 3	Tues/Wed April 10/11	**Microscopy:** 5. Calibrating your microscope: Demo and complete 6. Making a wet mount and Phase Contrast Microscopy: Wet 7. mounts and phase contrast:- view, identify, and measure (all with Hay Infusion)	**HW2 due – table**

		Understanding dilutions: 1. Understanding dilutions- theory **Measuring microbial growth: Yeast** ***2.*** Direct counts using a hemocytometer ***3.*** Using a spectrophotometer Counting viable cells using plating	
Lab 4	Thurs/Fri April 12/13	**Microscopy:** Continue/complete all wet mounts (all other bacterial and yeast) **Microsopy: Staining** 1. Smear preparation and simple staining 2. Gram stain: control organisms only **Characterizing the Test Organisms:** **Introduction**: Receive 2 test organisms and 2 unknown organisms per group of 4: make a wet mount, streak plate and set up broth culture with organisms **Winogradsky column** Understanding the set up, a first look	
Lab 5	Tues/Wed April 17/18	**Characterization of the Test Organisms** Streak stock TSS slant, do wet mounts from both temperatures **Microscopy: Staining** **1.** Complete staining of designated Gram positive and Gram **2.** negative controls **Characterization of the Test Organisms** **3.** Gram stain **4.** MacConkey – inoculate along with known G+ and G- organisms 5. Sticky test along with known G+ and G- organisms	**HW3 due – paper**

		6. Endospore test – inoculate NSM **Macronutrient use – how organisms get energy to survive:** Introduction: Hydrolysis and use of large extracellular materials 1. Polysaccharides: Starch plates - inoculate 2. Proteins: Skim milk plates and gelatin deeps - inoculate 3. Lipids: Rhodamine plates - inoculate	
Lab 6	Thurs/Fri April 19/20	**Inoculation of control organisms (to create fresh stocks):** *4. Enterobacter aerogenes* *5. Escherichia coli* 6. *Proteus vulgaris* Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the urease test on Lab 7 **Data Analysis Workshop:**	**York 3010 confirmed for data analysis workshop. Initial lab work in lab**
Lab 7	Tues/Wed April 24/25	**Characterization of the Test Organisms** **1.** NSM – Complete **Macronutrient use – how organisms get energy to survive** 2. Polysaccharides: Starch plates - complete 3. Proteins: Skim milk plates and gelatin deeps - complete Lipids: Rhodamine plates – complete **Special metabolic functions: Test organisms only** • Indole production from tryptophan, catabolite repression – inoculate • Urease test – inoculate • Differential utilization of citrate by enterics - inoculate **How energy is produced – aerobic vs. anaerobic breakdown**	***MT 1 in lecture*** **HW 4 online tutorial due**

		of organic compounds 1. Acid and gas production from sugar fermentation – inoculate Methyl-Red and Voges-Proskauer – inoculate	
Lab 8	Thurs/Fri April 26/27	**Fundamentals of library research: 90 minute hands on workshop** **Characterization of the Test Organisms:** **How energy is produced – aerobic vs. anaerobic breakdown** **of organic compounds** 2. Acid and gas from sugar fermentation - complete 3. Methyl-Red and Voges Proskauer – complete 1. T-streak plate for fresh isolated colonies (for Cyto C and 2. catalase) **Special metabolic functions: Test organisms only** • Indole production from tryptophan, catabolite repression – • complete • Urease test – complete • Differential utilization of citrate by enterics – complete **Inoculation of control organisms (to create fresh stocks):** *3. Escherichia coli* 4. *Pseudomonas fluorescens* *5. Enterococcus faecalis* 6. *Staphylococcus epidermidis* Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the Cyto C and catalase tests on Lab 9 *7. Escherichia coli* 8. *Pseudomonas aeruginosa*	**Workshop in York 3010 confirmed** **Lab in usual location**

		Inoculate 1 TSS slant of each per aisle. These slants will be used for the controls for the nitrate test on Lab 9	
Lab 9	Tues/Wed May 1/2	**Characterization of the Test Organisms:** **How energy is produced – aerobic vs. anaerobic breakdown** **of organic compounds** • Oxygen requirements – inoculate thioglycolate tube • H_2S production – inoculate • Cytochrome C test – complete • Catalase test – complete • Nitrate reduction – inoculate **Motility** – inoculate plate and deep with test organism **Survival in extreme conditions:** • Low pH • High pH • Low temp • High temp • High salt 20% NaCl • Moderate salt 5% NaCl • **Control TSB broth** inoculate appropriate broth with test organism **Winogradsky column**: preliminary observations	***HW4 due – online library tutorial and quiz***
		Students come in on non lab day to check thioglycolate tube and Kligler iron deep	
Lab 10	Thurs/Fri May 3/4	**Characterization of the Test Organisms:** **How energy is produced – aerobic vs. anaerobic breakdown** **of organic compounds** • Oxygen requirements –complete	

		• **Nitrate reduction** – complete • H_2S production – complete test **Motility** – complete **Survival in extreme conditions:** **1.** Score growth/no growth in each tube **Soil Enumeration:** First lab period: • **Enumeration**: Serial dilution, plating on TSA, SDA, GAA, and MacConkey, minimal media + skim milk plates (casein hydrolyzers), TSA incubated at high temp (thermophiles), TSA pH4 (acidophiles/acidoduric). Incubation at assigned temperature • **Enrichment of soil organisms**: inoculation of TSB or TSB + low pH as assigned; incubation at assigned temperature **Additional Lecture and Review: Tues sections, Room 3010 York**	
		Non lab day: TAs set up subculture of enrichments: Tues/Thurs TAs - Sat and Mon; Wed/Fri TAs – Sun and Tues	
Lab 11	Tues/Wed May 8/9	**Soil Enumeration and Enrichment**: Second lab period • **Simple Enumeration**: Colony counts and calculations • **Extracellular degradation**: **Enumeration**: test differential media, count, and calculate **Enrichment**: serial dilution and plating; centrifuge cell suspension and freeze pellet • **Extreme conditions** **Enumeration**: Colony counts and	***Lab Report 1 due*** **Midterm 1**

		calculations **Enrichment**: serial dilution and plating; centrifuge cell suspension and freeze pellet **Nitrogen fixation: Free-living - Anabaena** • Inoculate BG11 and BG11-0 with *Anabaena*	
Lab 12	Thurs/Fri May 10/11	**Soil Enumeration and Enrichment**: Third lab period • **Extracellular degradation**: **Enrichment**: test differential media, count, and calculate • **Extreme conditions** **Enrichment**: Colony counts and calculations **Metagenomics**: First lab period • Step 1: Chromosomal DNA preps from frozen cell pellets from various soil enrichments • Step 2: Set up 16S rRNA PCR **Screening for Antibiotic Producers**: grid plates	**HW5 due- Dilution problem**
Lab 13	Tues/Wed May15/16	**Metagenomics**: Second lab period • Step 3: Run gel and evaluate PCR results • Step 4: Purify PCR product **Screening for Antibiotic Producers**: Identify antibiotic producers, measure ZOI **Evaluation of antibiotics by the Kirby Bauer method** Spread plates with standards and test efficiency of antibiotics **Characterization of a Test Organism:** Create elimination flow chart for identification of genera	***Midterm 2 in lab*** **Receive HW 6 worksheets** ***Computer lab – complete elimination tree for Lab Report 2***

		Non lab period: TAs run gel of purified PCR product and set up ligations (Step 5 of Metagenomics)	
Lab 14	Thurs/Fri May 17/18	**Metagenomics:** Third lab period • Step 6: Transform ligations and plate on selective media **Evaluation of Antibiotics by the Kirby Bauer Method** Measure ZOI, identify any resistant colonies ***Characterization of a Test Organism:*** • *Create elimination flow chart for identification of genera*	***Computer lab – complete elimination tree for Lab Report 2*** **HW6 due - worksheets**
Lab 15	Tues/Wed May 22/23	**Metagenomics:** Fourth lab period • Step 7: Select white colonies and streak out for sequencing **Growth curve experiment** Growth and graphing of *Vibrio natriegens*	**Work on HW 7 in lab, complete after lab**
Lab 16	Thurs/Fri May 24/25	**Transposon mutagenesis:** Lab Period 1 Step 1: Set up conjugation of *E.coli* and *Salmonella* **Yogurt:** Inoculate milk with starter yogurt **Winogradsky column:** Second observation **Metagenomics:** Analysis of sample sequence data and sample construction of phylogenetic tree– computer lab	**Room and computer lab?** **HW7 due – growth curve** **Lab Report 2 due**
Lab 17	Tues/Wed May 29/30	**Transposon mutagenesis:** Lab Period 2 • Step 2: Plate exconjugants for selection and counterselection • Save LB recipient control plates for later use **Yogurt:** measure pH, gram stain **Metagenomics**: Step 8: Begin/complete analysis of all sequences, construction of phylogenetic trees.	**Computer lab** ***Lab Report 2 due***

Lab 18	Thurs/Fri May 31/June 1	**Transposon mutagenesis:** Lab Period 3 • Count colonies and calculate transposition efficiency • Step 3: Screen transposants (mutants) for loss of function mutations **Nitrogen Fixation** 1. Free-living: *Anabaena*: check for heterocysts 2. Symbiotic*: Rhizobium:* Observe nodules **Plant Pathogen** **1.** Observe *Agrobacterium*-kalanchoe interaction **2.** **Metagenomics**: Complete analysis/discussion of all sequences	**Computer lab**
Lab 19	Tues/Wed June 5/6	**Transposon mutagenesis:** Lab Period 4 • Check screens • Streak out mutants on LB/TSA plate for future use **Winogradsky column** Observation and sampling Check out Review	**HW8 due – Post workshop library survey**
Lab 20	Thurs/Fri June 7/8	Midterm 3 will be held during lecture hours – begins at 7:30 am	***Midterm 3***
	Mon Jun 11	**Mon June 11th 1 pm (Mon of finals week) – Lab Report 3 due**	