

SIO 288/BIMM127
MARINE MICROBIOLOGY LABORATORY
3316 York Hall, Spring, 2010

This advanced microbiology laboratory course will include a field trip. Students and instructors will take an evening trip out to sea to obtain various types of samples for the isolation of novel groups of environmentally relevant microbes. The first part of the course will entail setting up a variety of different types of microbial enrichments in order to learn more about prokaryotic diversity. Students will also be coached during the beginning phase of the lab course to work on an independent project of their own design. Additional labs will highlight microbial taxonomy, motility, pigment production and protozoa-bacteria interactions.

This course will be team taught by Drs. Brahamsha and Palenik from the Scripps Institution of Oceanography Marine Biology Research Division. Rhona Stuart will be the Teaching Assistant. Lolly Almazan will organize the laboratory. No textbook will be required, but it will be useful for students to read parts of “Brock, biology of microorganisms” by Madigan, Martinko and Dunlap and Clark for many of the exercises. In addition, detailed protocols and references for each laboratory period will be provided by the instructors. The class will entail two five-hour lab periods per week, Tuesday and Thursday from 12:30 – 5:30 pm, and additional independent time outside of the scheduled lab time.

Prerequisites (despite what studentlink may indicate): any biology or biochemistry lab class or consent of one of the instructors.

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LAB SCHEDULE

Week 1:

Tuesday, March 30 (Brahamsha/Palenik)

- A. Course overview, lab safety
- B. Students start thinking about independent projects
- C. Media preparation, autoclaving, sterile technique and streak plates
- D. Winogradsky column set up, p. 53.
- E. Brightfield and phase -contrast microscopy

Thursday, April 1 (Brahamsha/Palenik)

- A. Staining and visualization of microbes
 - 1. Gram Stain (*Escherichia coli*, *S. aureus*), p. 82.
 - 2. Phase-contrast identification of spores (*Bacillus sphaericus*), p. 91.
- B. Enumerating bacteria (from seawater)
 - 1. Direct counts (DAPI and/or SYBR green), pp.83-87.
 - 2. Viable counts
 - Plating efficiency on different media (2216, SWT, TCBS)

Week 2:

Tuesday, April 6 (Brahamsha)

- A. Microbes in Motion: Swimming, swarming, gliding, p. 125.
- B. Coliforms (isolation and enumeration from different marine environments: Torrey Pines and San Elijo lagoons, Scripps beach, Children's pool), p. 116.

Thursday, April 8 (Palenik/Brahamsha)

Marine Science Floating Laboratory twilight cruise, 5:30 – 9:30 pm. pp. 103-107.
<http://www.horizoncharters.com/>

Week 3:

Tuesday, April 13 (Brahamsha)

- A. Perform direct counts of cyanobacteria and total microbes from cruise samples
- B. Plate out seawater, mud and plankton samples.
- C. Enrich for marine actinomycetes, p. 114.
- D. Enrich for marine cyanobacteria

Thursday, April 15 (Bartlett)

- A. Adaptations of microorganisms to extreme environments, p. 96.
Halophiles/psychrophiles/thermophiles/piezophiles/alkaliphiles

Week 4:**Tuesday, April 20 (Brahamsha)**

- A. Marine bacterial taxonomy, p. 108.
 - 1. Phenotypic characters (growth temperature)
 - 2. Use of Biolog in identification of bioluminescent bacteria
 - 3. 16S rRNA gene amplification
 - 4. Colony amplification

Thursday, April 22 (Brahamsha)

- A. Marine bacterial taxonomy
 - Run gel and prepare template DNA
 - Set up DNA and primer for sequencing reactions
- B. Student project considerations

Week 5:**Tuesday, April 27 (Palenik)**

- A. **Preliminary proposal due** (1 page)
- B. Fluorescence *in situ* hybridization in the visualization of specific microorganisms, p. 128.

Thursday, April 29 (Palenik)

- A. Microbes and Metals, p. 132.
 - 1. Role of siderophores in iron uptake.
 - 2. ARISA
- B. Analyse sequence data
- C. **Extremophile report due**

Week 6:**Tuesday, May 4 (Brahamsha)**

- A. **Project proposal is due, guidelines p. 127.**
- B. Additional enrichments
 - 1. Chemoautotrophs/gradient cultures, p. 134.
- C. Independent project work

Thursday, May 6 (Palenik)

- A. Additional enrichments
 - 1. Phytoplankton: Cyanobacteria, diatoms, dinoflagellates, picoeukaryotes
- B. Examination of past enrichments
- C. **Taxonomy report due**

Week 7:**Tuesday, May 11 (Brahamsha)**

- A. Observations on various enrichments. Discussion of Winogradski column results
- B. Independent project work

Thursday, May 13 (Palenik)

- A. Additional enrichments
 - 1. Respiration and adaptations of microorganisms to alternative electron acceptors. Nitrate reducers/denitrifiers, b) Sulfate reducers, c) dissimilatory metal reducers, d) Methanogens, p. 138.
 - B. Independent project work
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Week 8:

Tuesday, May 18 (Brahamsha)

- A. Open lab for individual project work

Thursday, May 20 (Palenik)

- A. Open lab for individual project work
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Week 9:

Tuesday, May 25 (Brahamsha)

- A. Discussion of assigned paper (to be determined) and how to write a scientific paper in ASM style
- B. Independent project work
- C. **ARISA report due**

Thursday, May 27 (Palenik)

- A. Bacteria-protozoa interactions
 - B. Independent project work
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Week 10:

Tuesday, June 1 (Palenik)

- A. Open lab for individual project work

Thursday, June 3 (Palenik/Brahamsha)

- A. Lab clean up
 - B. Hand in independent project report
 - C. Independent project presentations
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Grading:

- 1. Participation: 100 points (attendance will be taken every day)
- 2. Lab reports (3 total): 25 points each
- 3. Midterm proposal: 50 points
- 4. Independent project written report: 100 points
- 5. Independent project presentation: 25 points

Total = 350 points

Reference books:

Brock biology of microorganisms /, Michael T. Madigan, John M. Martinko, Jack Parker. 10th ed. Upper Saddle River, NJ : Prentice Hall, 2003 UCSD Bookstore.

Brock biology of microorganisms/Michael T. Madigan, John M. Martinko, Jack Parker. 9th edition. Upper Saddle River, NJ: Prentice Hall, 2000. UCSD BML QW4 M182b 2000. Class reserves

Brock biology of microorganisms/Michael T. Madigan, John M. Martinko, Jack Parker. 8th edition. Upper Saddle River, NJ: Prentice Hall, 1997. UCSD BML QW4 M182b 1997, UCSD Scripps QR41.2B77 1997

Additional reference material:

The Prokaryotes : a handbook on the biology of bacteria : ecophysiology, isolation, identification, applications /, edited by Albert Balows ... [et al.] 2nd ed. New York : Springer-Verlag, c1992. 4 volumes. Scripps Inst. Oceanography Library QR100 .P76 1992 Reference book

Bergey's manual of determinative bacteriology. John G. Holt et al. (ed.). Baltimore: Williams and Wilkins. 1994. UCSD BML QW 4 B496 1994

Manual of environmental microbiology (C. J. Hurst, G. R. Knudsen, M. J. McInerney, L.D. Stetzenbach, and M. V. Walter (eds.)). Washington D. C.: ASM Press. 1997.

