MICB 401: Environmental Microbiology Laboratory 2013W

I. Updated course description

MICB 401 (3) Environmental Microbiology Laboratory. Microbiological analysis of environmental samples using culture-dependent and culture-independent methods [2-4-0]. Prerequisite: MICB 322 and one of MICB 300, MICB 301.

II. Big picture

Understanding any microbial community means asking the following questions (Madsen, 1998):

"Who" is there?	How prevalent are "they"?	What are "they" doing?
DIVERSITY	**ABUNDANCE**	**ACTIVITY**

Diversity: Assessing microbial **diversity** means answering the question: What microbes are present and what are their characteristics?
Abundance: Assessing microbial **abundance** means answering the question: How "many" microbes are present in terms of **numbers** and/or **biomass**?
Activity: Assessing microbial **activity** means and answering the question: What are the microbes doing and as a result, how are they impacting the abiotic and biotic environment?

The MICB 401 laboratories and lectures explore ***some*** of the methods that environmental microbiologists use to assess the **diversity** and **abundance** of microorganisms in various environmental samples. Although the course does not specifically target the assessment of microbial **activity**, there will be opportunities to observe the activities of various microorganisms. It should be noted that although the terms "microorganism" and "environmental microbiology" are used in this introduction, MICB 401 focuses largely on those microorganisms which are members of the domain Bacteria.

III. Course goals (aims)

MICB 401 is a course about ***some*** of the methods used in environmental microbiology. At the end of the course, students should be to

- Explain how these methods work.
- Explain the information that can be derived from the use of these methods.
- Explain the strengths and weaknesses of these methods.
- Demonstrate proficiency in the execution of these methods in a teaching laboratory setting.
- Correctly interpret relevant passages in primary scientific publications that employ these methods.

IV. Emphasis and the MICB 401 laboratories

Methods used by environmental microbiologists can be grouped into two types: **culture-dependent** and **culture-independent**.

Culture-dependent: Any type of analysis that relies directly or indirectly on culturing to assess microbiological diversity or abundance.

Culture-independent: Any type of analysis that does not rely directly or indirectly on culturing to assess microbiological diversity or abundance.

Culture-independent analysis is generally synonymous with the analysis of DNA obtained directly from environmental samples. While this type of analysis (particularity as far as diversity is concerned) has eclipsed the culture-dependent variety over the last 20 years, it is now clear that both types of analysis are important and should be used together (See Donachie et al. 2007 for example). And quoting Stewart (2012, with minor edits) expressing a view held by many: "While molecular techniques, such as DNA sequencing, can provide some information independent of our ability to culture organisms, it is essentially impossible to learn new gene and pathway functions from pure sequence data. A true understanding of the physiology of bacteria and their roles in ecology, host health, and natural product production requires their cultivation in the laboratory."

The MICB 401 laboratories provide students with the opportunity to expore some laboratory strategies for both culture-dependent and culture-independent analysis of environmental samples. This being said, culture-dependent analysis dominates the course.

Diversity: Isolation of phototrophic bacteria- Students use anaerobic culture to isolate different types of phototrophic bacteria from sewage.

Diversity: Environmental DNA isolation and analysis- Students attempt to isolate PCR quality template DNA from soil using two different commercially available kits (PowerSoil, FastDNA for Soil) with very different protocols. Suitability of the template is tested by attempting to amplify bacterial 16S rRNA genes in the sample. If the template fails to amplify, students trouble-shoot until they successfully amplify the template.

Diversity: Isolation of endospore-forming bacteria-Students isolate an endospore-forming bacterium from soil; endospores are identified by phase contrast microscopy. This is followed by the preparation genomic DNA by lysozyme/SDS lysis-phenol/chloroform extraction-ethanol/salt precipitation, PCR amplification of the isolate's 3'16S-ITS-5' 23S rRNA gene region, TOPO-TA cloning and nucleotide sequence analysis to confirm the taxonomic placement of the isolate with the known endospore-forming bacteria.

Abundance: Biomass indicators-Students use fluorescence microscopy to estimate bacterial biovolume in a soil sample and the chloroform fumigation method to estimate the amount of microbial carbon in a soil sample. These biomass indicators are used to estimate bacterial and total microbial biomass in the sample.

Diversity: Patient cultivation of lithoautotrophs-Students attempt to isolate two types of slow-growing lithoautotrophic bacteria from soil.

Diversity: Isolation of luminous bacteria-Students isolate a luminous bacterium from a marine fish. This is followed by the preparation genomic DNA by a kit-based method, PCR amplification of a portion of the isolate's 16S rRNA gene, TOPO-TA cloning and nucleotide sequence analysis to confirm the taxonomic placement of the isolate with the known luminous bacteria.

Abundance: Microbial air sampling- Students monitor the abundance of culturable microorganisms in the air of the MICB 401 lab room over a 12 week period using the impingement method.

The methods explored in the MICB 401 laboratories are derived from recent, often practically-oriented, environmental microbiology research.

Do, Y. S., T. M. Schmidt, J. A. Zahn, E.S. Boyd, A. de la Mora and A.A. DiSpirito. 2003. Role of ***Rhodobacter*** sp. strain PS9, a purple non-sulfur photosynthetic bacterium isolated from an anaerobic swine waste lagoon, in odor remediation. Appl. Environ. Microbiol. 69:1710-1720.

Budsberg, K.J., C.F. Wimpee and J.F. Braddock. 2003. Isolation and identification of ***Photobacterium phosphoreum*** from an unexpected niche: migrating salmon. Appl. Environ. Microbiol. 69: 6938-6942.

Lipson, D.A. R. F. Wilson and W. C. Oechel. 2005. Effects of elevated atmospheric CO_2 on soil microbial biomass, activity and diversity in a chaparral ecosystem. Appl. Environ. Microbiol. 71: 8573–8580.

Dineen, S.M., R. Aranda, D.L. Anders, and J.M. Robertson. 2010. An evaluation of commercial DNA extraction kits for the isolation of bacterial spore DNA from soil. J. Appl. Microbiol. 109:1886–1896.

Nehme, B., V. Létourneau, R.J. Forster, M. Veillette and C. Duchaine. 2008. Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. Environ. Microbiol. 10: 665–675.

Wei, S., M. Sanchez, D. Trejo and C. Gillis. 2010. Microbial mediated deterioration of reinforced concrete structures. Int. J. Biodeterior. Biodegrad. 64:748-754.

Vaerewijck, M.J.M., P. De Vos, L. Lebbe, P. Scheldeman, B. Hoste and M. Heyndrick. 2001. Occurrence of ***Bacillus sporothermodurans*** and other aerobic spore-forming species in feed concentrate for dairy cattle. J. Appl. Microbiol. 91:1074-1084.

Soil often serves as a source of bacteria in the MICB 401 lab. Why soil? For the most part this is simply a matter of convenience and safety. A small amount of soil contains large numbers of diverse bacteria to work with. Further, the principles involved in working with soil as an environmental sample can be extended to other types of samples. For example, the principles involved in the isolation of DNA from soil

are the same as those involved in isolating DNA from water, gut contents, tissue or other samples. Parenthetically, it was the analysis of soil DNA that first led to the realization that large numbers of microorganisms in all types of environments have yet to be cultured (Torsvik et al. 1990).

V. Emphasis and the MICB 401 lectures

MICB 401 consists of a weekly 4 hr lab component and a weekly 2 hr lecture component. Lecture time is used to

- provide information concerning the methods used to assess the abundance and diversity of microorganisms in environmental samples including information about culture-independent methods students do not use in the MICB 401 laboratories.
- discuss the background information required for the current week's laboratories.
- provide an opportunity for students to resolve any questions they have about upcoming laboratory work.
- discuss the results obtained in previous week's laboratories.

VI. Student Evaluation

Course component	**% of Course Grade***
Practical work: (eg. Streak plates, wet mounts, slants, Gram-stains, smears etc)	35%
General laboratory performance	5%
Quizzes/Assignments	15%
Laboratory exam and Laboratory Notebook Data Retriveial Test (Last Lecture in class)	20%
Final exam (Date scheduled by Enrollment Services)	25%

*** This is tentative and may have to be modified depending on results obtained in the laboratory.**

VII. References

Donachie, S. P. et al. 2007. Culture clash: challenging the dogma of microbial diversity. ISME J. 1:97–99.

Madsen, E.L.1998. Epistemology of environmental microbiology. Environ. Sci. Technol. 32:429-439.

Stewart, E. J. 2012. Growing unculturable bacteria. J. Bacteriol. 194:4151-4160.

Torsvik, V. et al. 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56:782-787.