MICB 401 COURSE INFORMATION 2019W T1

I. COURSE CALENDAR 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.

Note: Although MICB 401 is entitled "Environmental Microbiology Laboratory", the course focuses on prokaryotic microorganisms which are members of the domain Bacteria and to a lesser extent the Archaea.

II. PREREQUISITES AND PREVIOUS LABORATORY EXPERIENCE

The prerequisites for MICB 401 include MICB 322 and MICB 300/1 which in turn require BIOL 201 and MICB 201. Students are **responsible** for transferring the relevant parts of these courses to MICB 401. **This responsibility extends to examinations.**

It is **expected** that students have some basic microbiological laboratory experience including:

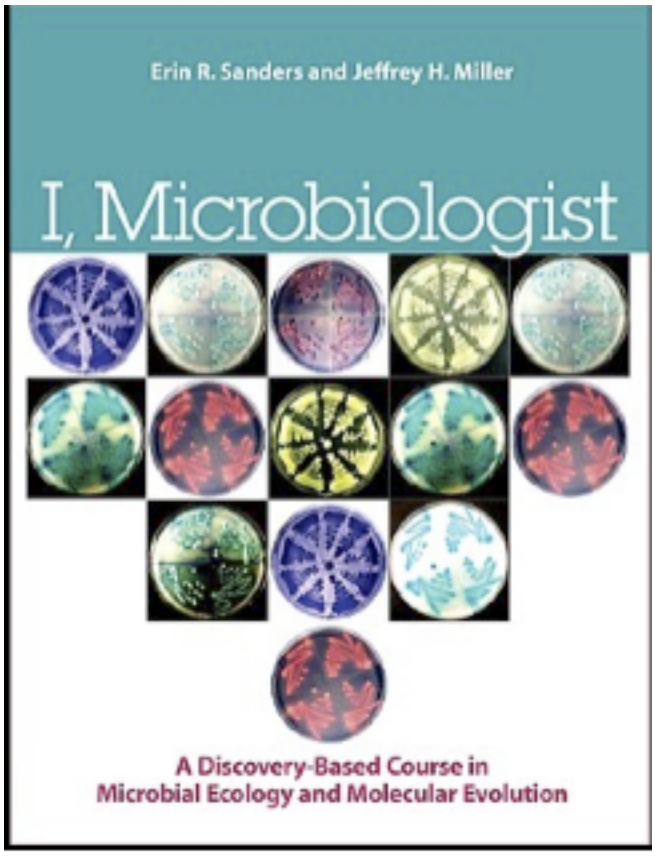
- Laboratory safety and citizenship
- Pipetman operation
- Aseptic technique
- Inoculation of microbiological media
- Incubation of microbiological media
- Basic laboratory calculations involving amounts and concentrations
- Bright field light microscope operation
- Microcentrifuge operation
- Gram stain theory and preparation
- Serial dilutions, theory and preparation
- Agarose gel electrophoresis theory and practice
- Spectrophotometry as applied to solutions and suspensions
- PCR theory and practice, Q-PCR theory
- Plasmid cloning using ***E. coli*** vectors, chemical transformation of ***E. coli,*** transformant selection, and plasmid isolation from ***E. coli*** using alkaline lysis methods.

If a student is unsure about their competence in any of these areas, it is their **responsibility** to clarify any ambiguities by consulting the relevant references, a TA or the instructor. This is important because basic theoretical and practical laboratory competence is expected not only for day-to-day work but also for evaluation purposes.

III. THE BIG PICTURE

A. "I Microbiologist" and soil

The current version of MICB 401 was inspired by "I Microbiologist: a Discovery-Based Undergraduate Research Course in Microbial Ecology and Molecular Evolution" by Erin Saunders and Jeffery Miller (2010) which concerns methods for analyzing **soil microbial communities**.



Understanding any microbial community begins with asking the following questions:

Who's there?	How prevalent are they?	What are they doing?
DIVERSITY	**ABUNDANCE**	**ACTIVITY**

Diversity: What microbes are present and what are their characteristics?

Abundance: How "many" microbes are present in terms of **numbers** and/or **biomass**?

Activity: What are the microbes doing and as a result, how are they impacting the abiotic and biotic environment?

Like “I Microbiologist”, soil serves as an environmental sample for the MICB 401 laboratory projects. 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 microorganisms to work with at little risk to the curious investigator if proper aseptic technique is used (Trevors, 2010; Fierer, 2017). Further, the experience gained by 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 samples, the contents of the human gut and other samples. Indeed, it has been said “"If you can get DNA out of soil, you can pretty much get it out of anything.” (Weaver, 2013). Parenthetically, it was the analysis of soil DNA that first raised the possibility that large numbers of microorganisms in all types of environments had yet to be cultured (Torsvik et al. 1990).

B. Culture-dependent and culture-independent methods

As the MICB 401 calendar description indicates, the methods used by environmental microbiologists to analyze microbial communities are often grouped into **culture-dependent** and **culture-independent** ones.

- **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. Although culture-independent analysis is generally synonymous with the analysis of DNA obtained directly from environmental samples, it does not have to be.

Culture independent methods (particularly as far as assessing diversity is concerned) have dominated microbial community analysis for the last 25 years. This reliance on culture-independent methods has often been justified by asserting that most prokaryotes ***cannot*** be cultured because suitable culture conditions ***cannot*** be established in the laboratory, While it is indisputable that the vast diversity of prokaryotes have not been cultured, there is no convincing evidence that most prokaryotes ***cannot*** be cultured in the laboratory (Gest, 2008 a,b,c) In fact, so-called “unculturable” prokaryotes are being cultured all the time not only from the soil microbiome but also from the human microbiome (eg. Browne et al. 2016).

MICB 401 mostly concerns culture-dependent analysis. This emphasis should not be taken to mean that these methods are superior to culture-independent ones. Indeed, culture-dependent methods can never hope to catalogue the huge diversity of the prokaryotic world. For this, culture-independent methods are indispensable. The balanced view is that both types of analysis have their strengths and weaknesses and for this reason should be used together (Anonymous, 2013; Marx, 2017).

IV. MICB 401 LECTURES AND LABORATORIES

The MICB 401 laboratories and lectures explore ***some*** of the methods that environmental microbiologists use to assess microbial community **diversity**. At the end of the course, students should be able 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.

Although the course does not specifically target the assessment of microbial abundance or activity, there will be opportunities to observe the activities of various microorganisms.

A. Laboratories

The current MICB 401 projects are

I. More than dirt
II. Survival of the fittest-I
III. Survival of the fittest-II
IV. So many kits
V. Hunting the wild pseudomonas
VI. All washed-up

The MICB 401 laboratories mostly concern culture-dependent analysis. This emphasis should **not** be taken to mean that these methods are superior to culture-independent ones. Indeed, culture-dependent methods can never hope to catalogue the huge diversity of the prokaryotic world. For this, culture-independent methods are indispensable. The **balanced** view is that both types of analysis have their strengths and weaknesses and for this reason should be used together (Marx, 2017).

Although the start date for particular laboratory work can be predicted with certainty, often how a project proceeds week-to-week cannot. This is because much of MICB 401 involves working with uncharacterized environmental isolates which cannot be "counted on" to grow according to a university schedule. In addition to the lack of predictability about the content of any particular laboratory session also consider the following

- Usually the work associated with several projects will be on-going at the same time.
- Much of the laboratory work involves lengthy procedures in which the success of one day's work is dependent on the successful execution of the previous day's work.

Clearly without satisfactory preparation and time management, the MICB 401 laboratory has the potential to become a confusing experience requiring an inordinate amount of time to complete.

There are some occasions when students will be **expected** to come in outside of the scheduled laboratory session (eg. to inoculate cultures or make subsequent observations). The time required for

these procedures will be compensated for by those occasions in which the laboratory work is completed early during regularly scheduled laboratory periods. Note that students are **expected** to do their own work, not send another student to do it for them.

B. Lectures

MICB 401 lecture time is used to

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

For these reasons, students should bring both their laboratory records and their Laboratory Handbook to lecture class. Indeed, a student's laboratory notebook and their MICB 401 Laboratory Handbook may be collected at any time without notice for spot checks.

V. GRADING

Term work*	45%
Final exam (Date scheduled by Enrollment Services)**	50%
General laboratory and lecture performance***	5%

* A breakdown of term work will be provided during the term. There is **no** midterm exam.
** A final examination of 2.5 hours duration will be held during the December final exam period. This exam will evaluate a student's understanding of the culture-dependent and culture-independent methods used and/or discussed in the MICB 401 labs **and** lectures. More detailed information about the content of final exam will be provided during the term. During the final exam, students are allowed to consult their MICB 401 Laboratory Handbook as well as their laboratory records in the form of a hand-written laboratory notebook. For this reason, students should benefit from keeping organized laboratory records that stress important information in a legible, organized and succinct manner rather than illegible, disorganized records that focus on trivial information (Suggested guidelines for keeping a laboratory notebook in MICB 401 are given in the MICB 401 Laboratory Handbook). Note that most laboratory sessions are designed so students have time to complete their laboratory records by the end of the session. Of course, it is up to each student whether they choose to use this time or leave the laboratory early.
***Criteria posted on Canvas.

VI. REFERENCES

Anonymous. 2013. The cultural revolution. Nat. Rev. Microbiol. 11:1.

H. P. Browne, H.P., S.C. Forster, B. O. Anonye, N. Kumar, B. A. Neville, M. D. Stares, D. Goulding and T. D. Lawley. 2016. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature 533: 543-546.

Fiera, N. 2017. Embracing the unknown: disintangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15: 579-590.

Gest, H. 2008a. The Modern Myth of "Unculturable" Bacteria/ Scotoma of contemporary microbiology (http://hdl.handle.net/2022/3149).

Gest, H. 2008b. Scotoma in contemporary microbiology. Microbiololgy Today, November 2008: 220.

Gest, H. 2008c. Unknowledgeables promote "unculturables". Microbe 3: 499.

Marx, V. 2017. Microbiology: the return of culture. Nat. Meth.14: 37-40.

Torsvik V., J. Goksøyr, and F. L. Daae. 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56: 782-787.

Trevors, J.T. 2010. One gram of soil: a microbial biochemical gene library. Annonie Van Leeuwenhoek 97: 99-106.

Weaver, J. 2013. DNA extraction: Overcoming obstacles in microbial studies. (http://www.biotechniques.com/news/DNA-Extraction-Overcoming-Obstacles-in-Microbial-Studies/biotechniques-335698.html)