

Study Guide
MICROBIAL DIVERSITY
Brock Ch. 18

This study guide covers Brock Ch. 18: methods in microbial ecology.

1. What is microbial ecology?
 - a. What are the two main branches in microbial ecology? (List, define)

2. Define **enrichment**.
 - a. What are the two key characteristics of the media or conditions used in enrichment culture technique?

 - b. List one major advantage of this method (and all culture-based methods), and one major disadvantage of this method (and all culture-based methods).

 - c. In addition to optimizing the culture medium, what else must you optimize when using the enrichment culture technique?

3. We met the **Winogradsky column** when we talked about the domain bacteria. Define the Winogradsky column and familiarize yourself with (1) the methods used to construct one, and (2) one of the major advantages of doing so.

4. Above, you were asked to state a disadvantage of the enrichment culture technique. Such problems are described in some detail starting on p. 618. Let's explore one of these problems (enrichment bias) a bit more. Enrichment bias can be demonstrated by comparing **dilution methods**. Explain.

As a note, there are other biases, too: what if we don't know what conditions to optimize to capture a particular species in culture? Think about all of those phyla represented only by SSU rDNA sequences. We have a long way to go before we figure out how to isolate them using the enrichment culture technique.

5. Following enrichment culture, pure cultures can be obtained in several ways. You are probably familiar with the **streak method**, in which small amounts of a particular bacterium are repeatedly spread across an agar plate until a pure culture is isolated. In this case, the bacterium of interest is present on the surface of the agar plate.

Briefly describe the **shake-tube method**. What organisms can be isolated this way?

Briefly describe the **serial dilution** method, also known as the **most probable number** method. What is one strength of this method?

Finally, some researchers use the so-called **laser tweezers** method. This method uses the force of a focused laser to move individual cells away from others, which allows those cells to then be flushed into a new medium, and/or replicate in the absence of competitors. This method is important for isolating bacteria that grow slowly relative to others.

6. Sometimes, researchers in microbial ecology are more interested in enumerating (counting) particular microbes, rather than isolating them. There are two ways to do this: staining, and molecular analysis of communities. We'll deal with staining methods first.
 - a. What does **DAPI** stain bind to? Name two advantages and one disadvantage of this method.

 - b. **Viability staining** is useful because it allows one to distinguish between...

But one disadvantage of this method with regard to environmental samples is...

- c. **Fluorescent antibodies** are highly _____. This is the main advantage to this method; the disadvantage is that specific antibodies must be prepared, which can be time- and labor-consuming.

- d. **Fluorescent protein**: under this method, bacterial cells are genetically altered so that they fluoresce. This is done with GFP, or green-fluorescent protein, which can be inserted into the bacterial genome and expressed. This method can be used in specific habitats (e.g., plant roots). Note that this is **not a staining method, per se!** **Nothing is stained; instead, the bacteria are transformed so that they express a protein that is visible to us.**

Note that these are all important methods. However, there are some major limitations to microscopy-based approaches to enumerating microbes. Clearly state two limitations below.

7. The next class of methods refers to **nucleic acid probes**. What are these, and how do they work?

Nucleic acid probes are the basis of FISH – fluorescent in situ hybridization. The chapter discusses three methods of FISH. State the target of the probe for each method listed below.

Phylogenetic staining using FISH

Chromosome painting

In situ reverse transcription

Why might ISRT be more useful in ecology than CP or Phylogenetic staining?

8. The next class of methods focuses on identifying microbes using molecular tools -- without culturing or directly observing those microbes.

a. What is the general method here, and what is the rationale?

b. Remind yourself of the major steps in PCR.

c. Remember that 16S = SSU rDNA (16S is traditionally used when referring to Bacteria).

- d. Carefully examine Fig. 18.14. This should remind you of our earlier discussion in class regarding ways to sequence SSU rDNA from environmental samples. **Make sure that you can reproduce this figure for Exam 3.** Note that at the bottom of the figure, there's the path that we took in class (cloning), and another: DGGE.
- i. What does DGGE stand for? What is it?
 - ii. What do the different bands in a DGGE analysis correspond to?
 - iii. Why is this separation step very important when characterizing microbial communities?
 - iv. What is one major lesson learned thus far from PCR-based exploration of microbial communities (both via DGGE and cloning)?
9. Now, we'll turn to measuring microbial activities in nature. There are three major ways that this work is done: using radioisotopes; using microelectrodes; and using stable isotopes. One **major disadvantage** here is that we don't know who's doing what – we only can measure the collective microbial activity of the entire community.
- a. In some cases, direct chemical measurements can be used to examine microbial activity. For a more sensitive analysis, **radioisotopes** are used. For example, if one were to study photosynthesis, one might place radiolabelled CO₂ into the microbial community, and could compare its concentration under light and dark conditions. Controls are very important in this work.
 - b. In some cases, **microelectrodes** are used. In this case, tiny electrodes are moved by micromanipulators to particular places or depths in a sample. These are frequently used, for example, in the study of stratified ecological roles in microbial mats (different activities at surface, interior of mat, base of mat), even though the mats may be only a few millimeters thick.
 - c. **Stable isotopes** are used in the third set of methods. What are stable isotopes?
 - i. What is meant by **isotopic fractionation**?
 - ii. Isotopic fractionation can reveal the biological origin of particular substances. How can ¹²C/¹³C composition of a substance reveal its origin?
 - iii. How have stable isotopes been used in the argument regarding the potential occurrence of life on the Moon?