Exploring Native Microbiota Lab Manual

BIO398, Winter 2024

Lab 1 : An Introduction to Environmental Sampling of Microbes

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Background:

Your task in this course is to obtain microbial samples from an environment of your choice. While the methods that students employ will be as diverse as the questions you ask, some general principles apply. There are two key points to take into account:

- microbes are everywhere, and
- microbes are very sensitive to changes in environmental conditions

The former point (microbes being everywhere) makes microbial sampling *both easy and hard*. Easy because you don't have to go far to sample something interesting. Hard because your samples are easy to contaminate. Fastidiousness during sampling is important to avoid contamination. What kind contamination? Microbial contamination of course. This could be live cells from your body or your sampling kit, but it could also be DNA! PCR is an extraordinarily sensitive technique so even small amounts of DNA contamination can compromise your samples, especially if you are sampling small amounts of material. Fortunately, there are simple ways to avoid this kind of contamination.

The latter point is one that may not be obvious to those who haven't worked with microbes before. If you sample a lobster, you don't really have to worry about it reproducing in the time between picking it out of the ocean and bringing it back to the lab. However, with microbes, this is a real concern! Some microbes (e.g. *Vibrio cholerae, E. coli*) can divide every 20 minutes. So, even an hour at room temperature could cause major changes in your samples, and you would have no way of knowing until you got your data back (and even then it might not be clear). In addition, when you sample you will be shocking the microbes by taking them outside of their natural environment. Since we want to preserve these cells for future analyses, it's important to minimize this disturbance.

Note on sample types: This is an environmental microbiology course, and I do expect that most samples will be taken from the natural environment, i.e. not from human-associated sites (e.g. your house, your pet's food bowl, etc). The rationale is that you are much more likely to discover something interesting this way, and contribute to broader scientific knowledge vs. more well-studied human systems. That being said, I may make some limited exceptions if you have a really compelling scientific reason to do so. Please discuss with me if this applies to you!

Also, environmentally-derived samples that are associated with macroorganisms (e.g. plants or animals that are wild) are fine, but you have to be careful to separate the microorganisms from the macroorganism. Otherwise, your data will be dominated by the host, and you'll miss the symbionts. This is because the primers we use amplify both 18S SSU rRNA (what we and other eukaryotes have) as well as 16S SSU rRNA (what prokaryotes and prokaryote-derived organelles have).

Lab and field safety:

In this course, you have an enormous amount of freedom to do what you want. However, this means you are also responsible to work with me to ensure your own safety, as well as those of your classmates. To paraphrase Spiderman, "With great freedom comes great responsibility".

One of the most important principles of safety is to identify hazards ahead of time. There are several categories of hazards you will encounter in this course (arranged in likely order of danger):

- Environmental hazards (heat, cold, tripping, slipping, water, hazardous chemicals, wildlife, extreme weather, etc.)
- Laboratory equipment hazards (centrifuges, extreme cold, razor blades, bunsen burners, etc.)
- Laboratory chemical hazards (ethanol, linear acrylamide, liquid nitrogen, etc.)
- Biohazards (your samples could potentially contain pathogens)

As a result of the diversity of potential hazards, it is critical that you discuss with me your sampling plans & proposed processing methodologies so we can figure out what potential hazards exist and how to mitigate them. I will also discuss some general principles of Biosafety that are relevant to working in a BSL-2 lab (same level as Biology 315), as well as go over these hazards with you in this first lab.

Note: At this point, you needn't worry about the DNA extraction and PCR steps. I will have a general protocol that should work on whatever type of sample you throw at it.

General principles of microbial sampling:

The most important thing is to do your best, be safe, and not to panic. The nice thing about field sampling is that you go out, do your best, and then move on with your life (in contrast to laboratory experiments)! That being said, I think it makes sense to outline a few basic rules to ensure success:

1. *Prepare more than you think you need to before sampling*. When you are in the field, it will be a lot more challenging than you think (especially during winter months if you are sampling outdoors).
2. *Take more samples / volume / weight than you think you will need*. Things go wrong. Samples get lost. DNA extractions fail. You want to have backups if possible.
3. *Be as clean as is reasonable for your field site*. If possible, wear gloves while sampling, use clean tools / sampling containers, and bring some ethanol and tissues to clean tools between samples. Basic household items such as aluminum foil can be great for making a nearly-sterile / DNA-free location.
4. *Keep things cold after sampling*. This prevents microbial growth and/or breakdown of the DNA/RNA you want to analyze.
5. *Process or freeze samples as soon as possible*. In some cases, it would be best to freeze samples immediately but this will most likely not be possible for samples for DNA. I will provide you

with icepacks that have been kept at -80°C. These can be used to freeze small volumes of liquid, and will be safer/cheaper than using liquid nitrogen or dry ice. Otherwise, you should store materials at either -20°C (fixed samples for FISH, filtrate for nutrients) or -80°C (cells, samples for nucleic acid extraction).

Specific considerations for the long-term use of your samples / datasets:

This course is inspired by my undergraduate experience sampling vascular plants. With plants, it is common practice to preserve specimens by pressing and drying, which can then be stored in a sample library a.k.a., a "herbarium". This herbarium can then be used in the future as a reference. Perhaps it could help tell us how plant populations are changing due to climate change, or some of the biomass could be analyzed using chemical/biological techniques. One of the goals I have for this course is that samples collected by students year after year should be available for analysis and follow-up by researchers (you or others).

However, many scientific samples are not really usable by future researchers because they lack important associated data. In many cases, this is really basic stuff. Where were the samples taken (e.g. GPS coordinates, lat/long), elevation, and at what date/time? What method was used to obtain the samples? Can the samples in the freezer be identified based on the labels you wrote?

Aside from these basic things: chemical, physical parameters are really useful and helpful. Ideally, you should think about the project you plan to propose for your final OOI project and work back from there. That being said, you can cover your bases by taking a few basic parameters such as temperature, salinity, pH, and possibly others such as oxygen, nutrient levels, etc. Discuss with me if you think of a particular analyte you want to measure and we can see what we can do with departmental knowledge / resources.

Finally, don't forget about macroscopic characters and observations. Smells, shapes, context! Digital images can be really really helpful to remind you in the future about what you were doing when you took your samples.

Lab and Departmental Resources, Field Trips:

- You are welcome to use most anything in the lab (much of it is hand-me-downs from Lori Graham), but you must ask first, and sign it out (if it is reusable)
- You can borrow sampling equipment from the department (I will show you where to go for this) but you must sign it out and return in a reasonable timeframe
- We are scheduled to do a field trip next week, though it will probably have to be an indoor or campus field trip due to winter weather conditions

Lab 1 Activity: Develop a Sampling Plan

What environment would you like to sample? This is just for a thought experiment, so it does not need to be your final choice.

What would you like to observe? (e.g. microbial diversity across chemical, depth, spatial, temporal scale, or a specific type of organism, interactions)

What would your sampling matrix be? Soil, sediment, water, porewater, plant/animal tissue, etc?

What equipment would you need? Think both big (e.g. shovels, coring device, hip waders) as well as small (scalpels, spoons, filtration devices, etc).

What chemical, physical, or other data/observations would be needed alongside your microbial samples in order for your microbial community composition to be interpretable?

Propose a general procedure. Try to write out all the details (even if it seems redundant), and prepare it to present to your peers during lab.

Example sampling sheet for field sampling

Fill in all sections with a "*", and as many others as possible, using the following naming scheme: YY(MMDD)-Initials-S(ite)##-L(ocation)##-analysis.

For example, "240111-JM-S01-L01" would be the first site I sampled on Jan 11th 2024, and the first location at that site. You would append the analysis type (see below) at the end for each separate tube, and you can shorten the date to YY(season) if you don't have enough space on the tube. e.g. "24w-JM-S01-L01-DNA". This may seem a bit overkill, but it will allow someone to know what your sample is when they pull it out of the freezer 20 years later.

Site:_____ Date & time:_____

Sample name: _____ Location (GPS or lat/long):_____

Elevation:_____

SampleID	Temp (°C)	Salinity (ppt)	Notes

Basic analysis types (you can define others, just make sure to record what they mean):

DNA = Unpreserved filter, sediment, biomass, for subsequent DNA extraction. Store on ice in the field, process in lab ASAP, or freeze at -80°C prior to extraction.

cells = Whole, unfiltered liquid from site preserved with glycerol or DMSO for future cultivation attempts. Frozen on site or kept on ice until returning to lab.

FISH = Whole, unfiltered liquid from site, preserved with formaldehyde, and kept frozen at -20°C for possible future fluorescence *in-situ* hybridization analysis

chem = 0.2µm-filtered liquid from site, kept at -20°C until analysis. If analysis is to be done within a few days (e.g. pH reading, refractometer reading), it's OK to keep at 4°C.

Site:_____ Date & time:_____

Sample name: _____ Location (GPS or lat/long):_____

Elevation:_____

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