Activity 1: Indescribable

Based on video and online text content

30 minutes

Setup

"...there's this huge diversity out there that has never been described. More than 90 percent of the diversity we see in the environment we've never described." So says Dr. Anna Louise Reysenbach, whose job as a microbial ecologist is to collect and investigate microorganisms. This exercise will explore the challenges of finding and characterizing diverse microbes.

Working in pairs, use the terms explained in the Microbial Diversity online text chapter to describe the metabolism and growth conditions of several microbes that will be highlighted in the video. Then, consider how a researcher who wanted to study them would use this information to cultivate them in a lab.

Materials

- One copy of the List of Organisms and Instructions per person (master copy provided)
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided)
- Tips and Suggested Answers

List of Organisms and Instructions

See Tips and Suggested Answers for answers to some questions.

Exercise 1:

1. First, familiarize yourself with the terms that describe the metabolism and growth conditions of microorganisms. Using the terms on the overhead, describe each organism listed below, based on the information that is provided about it.
    a. *Thermocrinus ruber* grows in hot springs. It oxidizes inorganic molecules for energy and fixes carbon dioxide to generate complex organic molecules.
    b. *Pseudomonas aeruginosa* is a soil bacterium that is an opportunistic human pathogen; for example, it can live in the mucus-covered lung tissue of a cystic fibrosis patient.
    c. *Acidithiobacillus ferrooxidans* uses acidic conditions to oxidize iron and sulfur.
2. Now consider how you would cultivate each of these microorganisms in the lab. List the general categories of ingredients you would use if you had to make culture medium on which to grow each these organisms. What approximate conditions of temperature, pH and O_2 levels would you provide?

3. Look at the culture medium recipes you generated in question 2. Would any of the organisms on the list grow in a medium other than its own?

Exercise 2:

1. One of the challenges for microbial ecologists like Dr. Anna Louise Reysenbach is cultivating novel microorganisms. Specifically, she says "…just try to second guess what these organisms like to eat from the environment! Consequently, we've only been able to actually grow less than perhaps 1 percent of all the organisms you see under the microscope."

    For each of the following environments, come up with ideas for medium and growth conditions you might use when trying isolate previously uncharacterized bacteria from these areas.

    a. cold ocean waters
    b. an area with acidic pollution
    c. Utah's Great Salt Lake
    d. a sewage treatment facility
    e. soil from your backyard
2. Researchers are often faced with the predicament of having to identify bacteria that are unculturable in the lab. What are some techniques one could use to survey or sample the microbial diversity without having to cultivate the microorganisms from an environment? How could novel gene sequences or gene products from unnamed species be identified or characterized?

3. Microorganisms that grow in hot springs or that oxidize unusual minerals are not pathogenic to humans, and they are difficult to cultivate. What reasons can you come up with for studying them? What practical applications can you think of for these microbes?

Table of Terms

Energy Sources	
Phototroph	Light
Chemotroph	Chemicals
Chemoorganotroph	Organic Compounds
Chemolithotroph	Inorganic Compounds

Carbon Sources	
Autotroph	Carbon Dioxide
Heterotroph	Organic Compounds

Growth Conditions	
Aerobe	uses oxygen as an electron acceptor
Anaerobe	uses a non-oxygen electron acceptor
Extremophile	tolerates extremes of pH, temperature, or salinity
Thermophile	grows optimally above 45°C

Exercise 1: Answers

1. a. *Thermocrinus ruber*: chemotroph, chemolithotroph, autotroph (or chemolithoautotroph), extremophile; specifically, a thermophile
   b. *Pseudomonas aeruginosa*: chemoorganotroph, heterotroph
   c. *Acidithiobacillus ferrooxidans*: chemolithotroph, extremophile; specifically, an acidophile
2. For all organisms, it might be necessary to experiment with different O_2 levels, pH, or temperatures.
   a. *Thermocrinus ruber*: water, oxidizable inorganic compounds (containing sulfur and iron, for example), high temperature, and CO_2
   b. *Pseudomonas aeruginosa:* water, organic molecules (like sugars and amino acids), neutral pH, room temperature up to human body temperature, O_2
   c. *Acidithiobacillus ferrooxidans:* water, compounds containing iron and sulfur in reduced forms, low pH, room temperature or cooler

Activity 2: PCR Demonstration

Based on video and online text content

60 minutes

Setup

In the first activity, you speculated on how one might survey the microbial diversity in an area, even if the resident microorganisms can't be cultivated in the lab. The video mentioned using PCR (polymerase chain reaction) as a way to detect microbial DNA in a sample. In this exercise, you will see how PCR can take a minute quantity of DNA—theoretically from a single cell—and generate enough DNA for identification and evolutionary analysis of the microbes in the sample.

PCR is simply in vitro DNA replication. The process is fundamentally the same as in cells: separation of DNA strands, priming from a short primer sequence, and semi-conservative replication by DNA polymerase. Work in teams of four to demonstrate the technique of PCR. If you are interested in seeing an animation of PCR, go to http://www.learner.org/channel/courses/biology.

Materials

- One set of PCR materials per four people. Each set contains:
  - two cardboard strips, 12 inches long and 2 inches wide, with a light-colored surface that can be written on
  - 10 cardboard strips, 2 inches long and 2 inches wide, with a light-colored surface
  - eight cardboard strips, 10 inches long and 2 inches wide, with a light-colored surface
  - 40 rubber bands that can fit around the cardboard strips
  - one roll of tape
- One black marker per person
- One copy of the Instructions per person (master copy provided)
- One copy of the Discussion Questions and Microbial Diversity Chapter Excerpt per person (master copy provided)

Instructions

1. First, read about the process of PCR in this excerpt from the Microbial Diversity chapter:
   To replicate DNA in vitro, PCR takes advantage of a special property of the molecule: the hydrogen bonds. These bonds, which bind the complementary strands of DNA together in a double helix, are broken at elevated temperatures (about 95°C). Each single-stranded piece of DNA (**ssDNA**) is then built upon to form a new, double-stranded molecule (**dsDNA**). To initiate this, short "primers"—specific ssDNA fragments called **oligonucleotides**—must anneal to complementary regions on the single-stranded DNA. Deoxynucleotides (A,T,G and C) and DNA polymerase are added and, in a process called primer extension, the complementary copy of the ssDNA fragment is built. The result is two double-stranded DNA molecules identical to the original. Repeating these steps thirty times can result in a 10^9-fold amplification of the original molecule.

   Careful thermal cycling is required for PCR to proceed. For the primers to anneal to the ssDNA fragments, the temperature is reduced to about 55°C. However, at this temperature the original complementary ssDNA fragments will begin to re-anneal with each other. A high concentration of primers, and the tendency of the shorter primer strands to anneal more readily, ensures primer binding. The temperature is then raised again to about 72°C for primer extension. Underscoring the importance of microbes, the thermophilic bacteria *Thermus aquaticus* is the major source of the heat-tolerant DNA polymerase, which catalyzes primer extension and facilitates PCR.

   In order to amplify a particular gene, specific primers, unique to that gene, are used. Two oligonucleotide primers (oligos) are constructed to flank a region of interest. One oligo will be complementary to a region on one strand of DNA, and the other oligo will be complementary to a region downstream on the homologous strand.

2. Next, have one person on the team make the target DNA sequence and the other three make primers:
   a. Target DNA: The two 12-inch cardboard strips represent the target DNA sequence. On each strip, write 48 nucleotides (one letter per quarter-inch). Note that they are complementary strands, so line them up as well as you can. The sequences are:

      top strand (cardboard strip 1)
      5′ TGATGCCGTA/AGTATTCGAT/AGCTTCTGAT/TGATTACCGT/AGCTCCGG 3′

      bottom strand (cardboard strip 2)
      3′ ACTACGGCAT/TCATAAGCTA/TCGAAGACTA/ACTAATGGCA/TCGAGGCC 5′

   Line up the strips so the sequences are complementary and anti-parallel, and bind the two strands (each cardboard strip) together with three rubber bands.

   b. Primers: The 10 two-inch cardboard strips represent the primers. These have the following sequences (written one letter per quarter inch):

      5′ TGATGCCG 3′

      3′ TCGAGGCC 5′

   Make at least 10 copies of each primer. Note that for demonstration purposes, these sequences are much smaller than ones that would normally be used in PCR.

3. Amplify the target sequence by PCR:

   Step 1: Denaturation, at 94°C

   The double-stranded DNA is split into two. To simulate denaturation, remove the rubber bands from the cardboard strips.

   Step 2: Annealing, at 55°C

   The primers anneal to complementary strands of the target DNA. To simulate this, arrange the primer cardboard strips to target the DNA cardboard strips that have complementary sequences. Wrap a rubber band around the two.

   Step 3: Chain Elongation, at 72°C

   DNA Polymerase fills in the missing sequence. To simulate this, securely tape a 10-inch cardboard strip to the primer. Fill in the missing nucleotides, using the complementary sequence of the other strand as a template. When you are done, wrap two more rubber bands around the strands. You have duplicated the target DNA sequence.

   Continue, with one person acting as the denaturer, one person acting as the annealer, and one acting as the polymerase. The fourth person is the thermocycler, who tells the other three when to carry out their respective activities. In the interest of time, the thermocycler can help the other three.

   Go through three cycles of the process. You should have eight copies of the target sequence after the three cycles. After completing the exercise, talk through the discussion questions with your teammates.

Discussion Questions and Microbial Diversity Chapter Excerpt

1. What are the similarities between PCR and cellular DNA replication? What is different?

2. Assuming an unlimited supply of primers and free nucleotides, how many copies of the target sequence would you expect to have after 10 cycles of PCR? After 20? After 30? (Note: After three cycles you had 2^3.)

3. What factors might limit the numbers you obtained above?

4. Look at your target sequence again. Imagine that you used primers with the following sequences:

   5′ GCCGTAAG 3′ and 3′ TGGCATCG 5′

   What section of the target DNA would be amplified? After three cycles, how many copies of the end sequences, not covered by the primers, would there be?

5. Explain how PCR is used in microbial ecology to discover new organisms, even if they cannot be cultivated in the lab. How can be it used to identify new organisms, and characterize them with respect to related organisms?

6. PCR uses a DNA polymerase called Taq polymerase (so-named because it comes from a bacterium called *Thermus aquaticus*). Why not just use DNA polymerase from a well-known laboratory bacterium like *E. coli*?

After answering these questions, read the rest of the excerpt from the Microbial Diversity chapter.

Studying Unculturable Microbes with PCR

Imagine yourself on a team studying archaea at a deep-sea hydrothermal vent at the Galapagos Rift (an area known for its hydrothermal activity). You've found a new microbe. What do you want to know about it? What metabolic class does the microbe fall within? Does it make certain proteins? How does it survive the volcanic heat? Traditionally, asking such questions involved growing microbes in the laboratory. Unfortunately, replicating the conditions in which many bacteria and archaea grow is very difficult. For this reason, only a small fraction (perhaps only as few as one percent) of the microorganisms in nature has been cultivated. To identify and compare unculturable organisms microbiologists have turned to molecular genetic techniques.

Polymerase chain reaction (PCR) is one technique for studying organisms that cannot be grown in the laboratory. When only a small quantity of DNA is available from a particular source, PCR can be used to amplify that DNA and produce billions of copies of a designated gene-sized fragment. The technique has many applications, including the amplification of DNA from crime

scenes, analysis of cancer genes, and identification of pathogens. When an environmental sample contains unculturable organisms, scientists can use PCR to generate copies of microbial genes suitable for comparison.

To replicate DNA in vitro, PCR takes advantage of a special property of the molecule: the hydrogen bonds. These bonds, which bind the complementary strands of DNA together in a double helix, are broken at elevated temperatures (about 95°C). Each single-stranded piece of DNA (**ssDNA**) is then built upon to form a new, double-stranded molecule (**dsDNA**). To initiate this, short "primers"—specific ssDNA fragments called **oligonucleotides**—must anneal to complementary regions on the single-stranded DNA. Deoxynucleotides (A,T,G and C) and DNA polymerase are added and, in a process called primer extension, the complementary copy of the ssDNA fragment is built. The result is two double-stranded DNA molecules identical to the original. Repeating these steps thirty times can result in a 10^9-fold amplification of the original molecule.

Careful thermal cycling is required for PCR to proceed. For the primers to anneal to the ssDNA fragments, the temperature is reduced to about 55°C. However, at this temperature the original complementary ssDNA fragments will begin to re-anneal with each other. A high concentration of primers, and the tendency of the shorter primer strands to anneal more readily, ensures primer binding. The temperature is then raised again to about 72°C for primer extension. Underscoring the importance of microbes, the thermophilic bacteria *Thermus aquaticus* is the major source of the heat-tolerant DNA polymerase, which catalyzes primer extension and facilitates PCR.

In order to amplify a particular gene, specific primers, unique to that gene, are used. Two oligonucleotide primers (oligos) are constructed to flank a region of interest. One oligo will be complementary to a region on one strand of DNA, and the other oligo will be complementary to a region downstream on the homologous strand.

Back home, after your trip to the deep-sea hydrothermal vent, you want to determine what genus of bacteria you have in hand. You can use PCR to amplify the gene for ribosomal RNA (the gene isolated and sequenced by Woese from so many organisms when he constructed his "Tree of Life"). Then, you can choose conserved regions of the rRNA gene for primers. With adequate DNA from PCR, you could sequence the gene and compare it with millions of known rRNA gene sequences using a computer database. (See the *Genomics* unit.)

Alternately, you might want to ask if a microbe carries out a particular form of metabolism. Given the DNA sequence for a protein involved in a particular metabolic strategy—photosynthesis, for example—you could construct oligos so that the presence of that gene could be detected using PCR.

7. Besides its use in microbe detection and forensic analysis of crime scenes, what other uses do you know of, or can you think of, for PCR?

To view the *Rediscovering Biology* PCR animation, go to http://www.learner.org/channel/courses/biology.

Activity 3: Biofilm Stars

Based on video and online text content

15 minutes

Setup

Although we think of bacteria as single-celled organisms, increasing attention is being paid to the multicelled biofilms formed by many types of bacteria. Biofilms are ubiquitous, so information about how they form and how they function is relevant to medicine, environmental science, industry, and commerce. Working in teams of three, read about biofilms in the Microbial Diversity online text chapter, and consider some applications for this information.

Materials

- One copy of the Microbial Diversity online text chapter per three people (available online at http://www.learner.org/channel/courses/biology)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Discussion Questions

See Tips and Suggested Answers for answers to some questions.

1. Make a list of places where you would encounter a biofilm on an average day. (Check the Microbial Diversity chapter for general ideas.)

2. List the medical implications of biofilm formation. Consider both biofilms that form in the body and outside the body. (Again, consult the text chapter for ideas.)

3. In 2003, Dr. Søren Molin and Dr. Michael Givskov's labs synthesized a drug that might be useful for treating infections of *Pseudomonas aeruginosa*, an opportunistic pathogen of cystic fibrosis patients. The drug was modeled on a compound from the seaweed *Delisea pulchra*. The drug did not inhibit the growth of bacteria in culture; however, it did help infected mice clear the bacteria more efficiently. How do you think this drug works? Why would a seaweed produce an anti-bacterial compound?

4. What might be some advantages of using a quorum sensing inhibitor to treat *P. aeruginosa* infections, instead of traditional bacteriostatic antibiotics that inhibit bacterial growth or bacteriocidal compounds that kill bacteria?

Source: Hentzer, M. et al. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J*. 22:3803–15.

Discussion Question Answers

3. How the anti-*Pseudomonas* drug works:

   The drug interferes with quorum sensing systems, so it prevents the formation of biofilms.

   Seaweeds do not have an immune system, but their tissues are just as susceptible as other organisms to degradation by bacteria. They produce the compound to protect against bacterial attack. To quote the article that describes this work:

   "…the Australian red macro-alga (seaweed) *Delisea pulchra* is largely unfouled in nature due to the production of biologically active halogenated furanones (de Nys et al. 1993). These secondary metabolites are released at the surface of the plant at concentrations that inhibit colonization by both prokaryotes and eukaryotes."

4. What might be some advantages of using a quorum sensing inhibitor to treat *P. aeruginosa* infections, instead of traditional bacteriostatic antibiotics that inhibit bacterial growth or bacteriocidal compounds that kill bacteria?

   When bacteria have formed a biofilm, higher doses of normal antibiotics are required to affect them because the cells in the biofilm protect each other from exposure to antibiotics. Some bacteria within the biofilm might be dormant and not as susceptible to the antibiotics. Quorum sensing inhibitors (QSI) prevent the formation of biofilms, so they do not have these limitations. QSI drugs inhibit virulence rather than growth, so they are less likely to provide a pressure that would select for resistance.

Activity 4: The Fall of Biosphere 2

Based on video and online text content

15 minutes

Setup

In the late 1980s, a glass-enclosed structure called Biosphere 2 was constructed outside of Tucson, Arizona. It was an attempt to create a sealed, self-sustaining ecosystem that included areas of ocean, desert, agriculture, and tropical rainforest, as well as humans. The human sustainability phase went from September 1991 to September 1993 and was terminated because of atmospheric irregularities. The structure is now used as a facility for research on climate change. In this exercise, read about the Biosphere experiment and discuss its successes and failures.

Materials

- One copy of the Discussion Questions per person
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided; see Activity 1)

Discussion Questions

1. The plant and animal components of Biosphere 2 were carefully considered, but the microbial components were unknown. Where do you think the microbial activity that altered the atmospheric conditions came from? What was the activity and how did it cause problems?

   After answering the question, read the following:

   > "…the rich soil was the major factor in causing the experiment to become unsustainable. Soil respiration was so high, and soil reserves of carbon were so great, that the atmospheric composition changed rapidly. Oxygen was absorbed from the air by soil microbes, and these released huge amounts of CO_2 from the soil back to the air. The buildup of CO_2 exceeded the photosynthetic capacity of plants to assimilate it and to regenerate O_2. While some of the excess CO_2 was absorbed by the fresh, unsealed concrete of the structure, forming limestone, the CO_2 concentration remained elevated above desired levels. More importantly, O_2 levels continued to decline rapidly, and additional O_2 had to be added to enable the eight human occupants to survive."

2. What types of microorganisms (chemotrophs, autotrophs, etc.) were responsible for the activities that changed the Biosphere 2 atmosphere? (Review the Table of Terms from Activity 1, if necessary.)

3. One solution might be to mix microbes with autotrophic activity into the soil. Do you think this would work? What might be some drawbacks to this approach?

   After answering this question, read the following from the same source:

   > "…the impact of disturbance whenever new soils are introduced results in large changes in physical structure of the soil (aggregation, peds, natural pores and cracks, horizon formation, fragipans and duripans) that develop over thousands of years in nature and simply cannot be reconstituted…such disturbances cause large increases in soil organic matter decomposition, which can cause a pulse of CO_2 and nitrogen release for up to several years. This is essentially the plowing effect, wherein wildland soils converted to agriculture typically lose 40 percent of their organic matter and nitrogen over time. This was the major reason that the first 'human experiment' failed in B2C."

   Another problem is achieving the proper balance of organisms. One problem involving macro-organisms is stated here.

   > "…Ants and other soil fauna are known to play a critical role in nutrient cycling, but all the terrestrial biomes are dominated by one exotic ant species that was able to survive while almost 20 other ant species originally placed in the biomes did not persist."

4. If you were to set up the next Biosphere project, what would you do differently?

5. What kind of parallels can you draw between the problems within Biosphere 2 and environmental problems in Biosphere 1 (which is earth)?

Source: U.S. Department of Energy. Biological and Environmental Research, Advisory Committee. *An Evaluation of the Biosphere 2 Center as a National Scientific User Facility*. March 2003.