Experiment 2: Pet microbes: A lesson in taxonomy
This experiment will start with three lab periods and then continue throughout the semester.

What's in a name? Names help us communicate a lot of information in a few words. They provide a shorthand to refer to individuals or groups of individuals. It is much easier to refer to "the Curie family" than it is to describe all of the characteristics of the family. A short name, such as Marie Curie, quickly communicates the identity of an individual who can be distinguished from most other individuals without a lengthy description of her attributes, when and where she lived, and what she did.

In biology, names of organisms communicate information as well. When we talk about *Homo sapiens* (human beings) or *Felis catus* (house cats), the two-part scientific name (or binomial) conjures up lots of information and images. Imagine how difficult it would be to communicate ideas without names. Without the precision of names, every time we wanted to talk about an organism we would have to describe all of the characteristics that distinguish it from other organisms.

Taxonomy, or the scientific classification of organisms, attempts to group organisms by their evolutionary relatedness. Therefore, a name communicates information about the organism's characteristics as well as what creatures are related to it. The binomial assigned to each organism contains a genus designation, which indicates the broad group of which it is a member, and the species designation, which indicates the specialized group of organisms that are similar in most respects. In higher organisms, members of a species can intermate and produce fertile offspring whereas members of different species cannot.

When we isolate an organism from nature, we must describe its properties to determine its name. If the organism has been described before, there may be a wealth of information about it. If it has not been described before, then finding out what microorganisms it is related to may provide some key hints about its role in the natural world. This means describing what it looks like, what its habits are, who it is related to. It also requires learning how we keep it alive. The aim of this project is to help you develop the skills to work with and name unfamiliar organisms.

Challenge

> In this project, everyone in the class will be issued their own nameless microbe to keep as a pet for the duration of the semester. It is your job to determine how to take care of and feed your pet microbe, to describe it, to determine its effect on other organisms, and to attempt to classify it. In addition, you must find out who else in the class has the same pet microbe. You will need to find a way to describe your pet microbe in a way that others will recognize it.

Resources

To familiarize yourself with the background relevant to this module, we've collected some online sources listed below. You can also find information in any microbiology textbook in the sections about (1) types of growth media (nutrients, selective and differential media), (2) isolation of pure cultures, (3) influences of environmental factors (temperature, salinity, pH, etc.), (4) procaryotic cell shape and structure (bacilli, cocci, Gram-negative, Gram-positive), (5) introduction to taxonomy, (6) microscopy.

Online resources:

- Todar's Online Textbook of Microbiology:
  - Nutrition and Growth of Bacteria http://textbookofbacteriology.net/nutgro.html
  - Important groups of procaryotes (overview on page 1) http://textbookofbacteriology.net/procaryotes.html
- Microbiology online lab textbook:
  - sections 2-1, 3-1, 3-5 to 3-10, 5-1 are particularly relevant http://inst.bact.wisc.edu/inst/index.php

In addition, protocols are posted that describe microscopy, preparation of slides and staining, and how to streak your pet microbe on an agar plate.

Pets throughout the semester

After the initial lab module (see below), you will continue to work with your pet throughout the semester. Remember, you must keep your pet alive all semester. This means transferring it to new growth medium once a week. Any day that you finish early, you are welcome to do an experiment with your pet. You are also welcome to include your pet in the experiments that we will run in other modules.

We will help you work with your pet microbes. Feel free to ask for help or materials to care for your pet microbe at any time. For example, if you need culture media, plant material, stains, or a microscope, just ask. We will do our best to get the materials that you need.

End of semester final report

A final report will be turned in at the end of the semester. This report will include:
1) a pure culture of the pet microbe
2) a description of the microbe
3) a discussion of the effect of the microbe on plants and other microbes
4) a classification of the microbe
5) a list of students who have the same pet

This lab is adapted from Handelsman J., Houser B., Kreiger H. *1997*. Biology Brought to Life. Times Mirror Higher Education Group, Dubuque, Iowa.

Pet microbe lab module (Jan. 18, 23, 25)

During the first three lab periods of this module, you will learn how to take care of your microbe using sterile technique and examine your microbe using microscopy. As part of this process, you will design an experiment to explore a growth property of your microbe. During the first class of this module, you will work in groups of 4 to formulate a hypothesis about the growth of your pets (you will have different pets, but the hypothesis should apply to all of them). You will make a prediction from the hypothesis and design an experiment to test the prediction. Reagents that you request for your experiment and controls will be provided on Jan. 23. Possible growth media for your experiment are described below. You will continue to investigate your pet through the remainder of the semester.

Module assessments

There will be two assessments for this module - the end of semester report described above, and an oral presentation during class on Jan. 25. The oral report will describe the experiment you designed and will be given collaboratively by your groups of 4. In 10 minutes or less, describe your hypothesis, the prediction, how your experiment was executed, the controls, the results, and your conclusions.

General growth media

On Day 1 of the pet microbe module, you will streak your pet microbe onto two types of growth media, nutrient agar (NA) and tryptic soy agar (TSA). NA supports growth of most non-fastidious bacteria (non-picky eaters). TSA supports growth of most bacteria. Your pet may grow equally well on both media or grow better on one medium than on the other.

Nutrient agar (for 1 liter)
3 g beef extract
10 g peptone
18 g agar
1 L distilled H_2O

Tryptic Soy Agar (for 1 liter)
3 g tryptic soy broth
18 g agar
1 L distilled H_2O
adjust pH to 7.2

Media for experiment on growth requirements of your pet microbe

You may request any of the following media to use for your experiment on Day 2.

1. **Salt: nutrient agar plates with 0.85%, 3.5%, 7.5%, 15% NaCl**
   Different types of bacteria have different tolerances for salt that are generally related to the salt concentrations encountered in their native environments.

2. **pH**: **glucose nutrient agar at pH 3, pH 5, pH 7, pH 9**
   Bacteria vary in their ability to grow at different pH (hydrogen ion concentration) values. Bacteria will usually grow across a range of pHs that are defined by the minimum, optimum, and maximum pH that can support growth. Acidophiles prefer acidic pH, neutrophiles prefer neutral pH, and alkaliphiles prefer basic pH.

3. **Secreted Proteases: Skim milk agar**
   Some bacteria secrete enzymes called proteases that degrade proteins. These extracellular proteases can be detected on a nutrient agar plate that contains skim milk. The major protein in milk is casein. Casein forms protein micelles (clusters) that give milk its cloudiness. If the bacteria secrete proteases that degrade casein, they will cut the proteins into amino acids and short peptides that no longer form micelles. As a result there will be a clear transparent area or halo around the streak of bacteria.

4. **Fermentation of mannitol and salt tolerance: Mannitol salt agar**
   Mannitol salt agar contains a relatively high concentration of salt, 7.5% NaCl, and the sugar mannitol. Only bacteria that can tolerate 7.5% NaCl can grow on this growth medium. If those bacteria can also ferment the sugar mannitol, they will produce organic acids that lower the local pH as a result of fermentation. The decrease in pH will change the color of the pH indicator, phenol red, that is in the growth medium from red to yellow. Bacteria that don't ferment mannitol will not produce organic acids so the area around the colony will be a reddish color (red-orange, pink or red).

Growth of Gram-negative bacteria and lactose fermentation

5. **MacConkey Agar**
   MacConkey agar is *selective* for the growth of Gram-negative enteric bacteria (bacteria that live in the digestive tract) and certain other Gram-negative species. Gram-positive species cannot grow on this medium, because they cannot grow in the presence of bile salts and crystal violet, which are included in this medium. Bile salts are produced by the liver and are present in the digestive tract, so gut-adapted bacteria and a few other Gram-negative species can tolerate them.
   MacConkey agar can also *differentiate* between bacteria that ferment lactose and those that cannot. As with mannitol, when bacteria ferment lactose, they produce organic acids that diffuse into the area surrounding the bacteria and lower the pH. The Neutral Red dye in the growth medium will turn red, and both the colonies and area around the colonies will be red. If the bacteria are strong lactose fermenters, they will produce a large amount of organic acids, lowering the pH to the point where the bile salts precipitate. The area around the colonies will be cloudy. Weak lactose fermenters do not produce as many organic acids, so the colonies will be red but the bile salts won't precipitate. Bacteria that can't ferment lactose at all will form white colonies.

6. **Eosin-Methylene Blue (EMB) agar**
   EMB agar is designed to *select* for Gram-negative bacteria. Growth of Gram-positive bacteria on this medium is inhibited by the dyes, eosin and methylene blue.
   Like MacConkey agar, EMB agar is used to *differentiate* between bacteria that ferment lactose and those that do not. The organic acids produced from fermentation lower the pH of the agar surrounding the bacteria causing the two dyes to combine and form a dark red-purple to blue-black precipitate. Bacteria that produce a lot of organic acids from fermentation will have a metallic green sheen, especially in the area of the plate

where the cells are close together (not isolated colonies). Species that can ferment lactose, but don't produce a large amount of organic acids, produce dark purple colonies but do not have the metallic green sheen. Bacteria that do not ferment lactose will not change the color of the surrounding medium and will be uncolored.