Appendix A

How to Culture Bacteria

Do you, as an amateur naturalist, need to culture bacteria? The positioning of these instructions in the appendix is meant to give a clear message: Culturing bacteria is an optional, extra step. You do not have to culture bacteria to appreciate them. If the process sounds like something you would rather not do in your kitchen, then don't do it! For those who decide to try some of these techniques, please read appendix B on safety. These projects are appropriate for enthusiastic but cautious amateurs.

A Little History

There are two major methods for culturing bacteria, each associated with a different tradition and philosophy:

1. *Pure culture methods*, in which the goal is to grow a single species of bacteria with no "contaminants" (other species) present
2. *Mixed culture methods*, in which assemblages of bacteria are provided with conditions as close to "natural" as possible so that they may grow and interact together

Pure culture has been predominant in Western microbiology and has been essential in the identification and characterization of bacterial species. Mixed culture methods were pioneered by Russians and until recently predominated in Russian microbiology; it is by means of these mixed culture methods that microbial ecological relationships have been deciphered and analyzed.

The pure culture methods outlined in this appendix are similar to the ones worked out in the late nineteenth century by the German

biologist Robert Koch, presumably with assistance from his wife, Emmy, as these procedures initially took place in their kitchen. Koch was eager to isolate single bacterial types so that he could determine the causes of certain diseases. He discovered that individual, distinctive colonies of bacteria would grow on the cut surface of a boiled potato. Other solid surfaces also gave good results, most notably, an aspic of gelatinized beef broth. Credit for this discovery is attributed to Lina Hesse, the wife of a coworker of Koch. Surely many other people (scientists and nonscientists) had noticed some sort of growth on aspics and other moist surfaces of food that had sat around uneaten for too many days. However, Koch (prompted by Hesse) was the first to recognize the practical significance of being able to grow individual colonies on hard nutrient surfaces. Modern Western microbiology was initiated using this technique and has been conducted in this manner ever since.

The mixed culture method was developed in the 1880s by the Russian microbiologist Sergei Winogradsky, who cultivated samples from the environment under controlled conditions in order to understand "the big picture." How are bacteria interacting with each other? How are they changing and using the chemicals in their environment? Thus began environmental and soil microbiology. The most famous method for mixed culture is called a Winogradsky column; instructions for making one are given at the end of this appendix.

Other methods of controlled mixed culture come out of culinary traditions such as sourdough baking, wine and beer making, cheese making, and pickling. Traditional agriculture also involves the (often naive) use of microbial communities in techniques such as composting and rotating fields with leguminous crops as well as maintaining healthy ruminant animals such as cows. The chapters on gram-positives provide several suggestions for making cheese, beer, compost, and so forth, though you should rely on other books for exact recipes and procedures.

Pure Culture Method and Sterile Technique

First, a disclaimer: these instructions will enable you to make as pure a culture as can readily be done in your kitchen. You most likely do

not have access to a high-pressure steam sterilizer (an autoclave) and therefore cannot keep your glassware and culture medium absolutely free of contaminating bacteria. These instructions call for boiling your medium and glassware, but you could use a pressure cooker; follow the instructions for the particular brand you have. Gram-positive bacteria and a few others can survive boiling temperatures and may turn up as contaminants; consider them to be interesting additions to your experiments. Indeed, you may consider survival of boiling a reasonably reliable indicator of the gram-positive nature of a bacterium.

Furthermore, you will not be using truly sterile (autoclaved or flamed) instruments but will take advantage of something well known to lab and field microbiologists. Packages of cotton swabs and toothpicks that have not been overly exposed to the air are, for many purposes, "sterile." Keep in mind, also, that bleach kills any microbe, so dousing with bleach will be your method of disposing of any cultures you do not want.

Abbreviated Procedure

Pour into "sterilized" (boiled) jars (e.g., baby food jars) $^1/_4$ to $^1/_2$ inch of hot "sterile" (boiled) dilute nutrient broth to which a solidifier has been added. Koch used gelatin; agar is used today in most microbiology labs and gives better results. Once the broth has cooled and is solid, you can use it to isolate and visualize bacterial colonies. Just swab on a sample using a cotton applicator or toothpick, put a boiled lid on, and wait a few days to see what grows.

Detailed Procedure

1. Boil 6 to 8 baby food jars and lids in a large saucepan or a pressure cooker and let them drain—while still hot—upside down on paper towels. Boil a 1 cup measure and a pint jar for mixing.
2. Decide on a source of nutrients. There are many choices—this is the creative step! In part, your decision should be based on an attempt to "think like a bacterium." Bacteria that are normally active in a particular environment tend to be rather specialized in their use of nutrients and other materials. Growing a specialized bacterium can sometimes tell you something about the microbial activity going on in the environment from

which you isolated it. Growing an opportunist or generalist is a little easier but is less creative and gives you less information. Opportunists respond well to rich, abundant food. Most likely they were resting in a dormant state until you provided them the food. Therefore, consider using a relatively dilute, specific medium to tease out the active microbial members of a community. Don't provide too much of a banquet.

Nutrients might include small quantities of soil or compost, dry or fresh vegetation, roots, feathers, manure (especially from herbivores), and even minerals. In all cases try amounts ranging from fractions of a teaspoon to a couple of tablespoons per 2 cups of water. You might also try to make a culture medium using water alone. There may be enough trace nutrients to sustain some interesting bacteria. Indeed, for photosynthesizers and chemosynthesizers, you want no nutrients at all; let them make their own food.

3. Choose your water. It could be tap water, pond or river water, or even distilled water. For marine bacteria, it could be ocean water or Instant Ocean from an aquarium supply store. If you use Instant Ocean, you can experiment with different salt concentrations.
4. Combine 2 cups of water plus a small amount of your chosen nutrient (if any) in a saucepan (or pressure cooker) and boil for a few minutes. How long? Some nutrients are destroyed by lengthy boiling, while others are released and become more accessible only after boiling. Simply experiment, perhaps starting with 3 to 5 minutes. If any particles remain in the broth after boiling, filter the broth through a coffee filter and boil a little more. Alternatively, don't boil but steep the nutrient in freshly boiled water (covered) for minutes to hours.
5. Choose either agar ("seaweed gel") from the health food store or gelatin from the grocery store to solidify the broth. Agar is preferable, although it may be more expensive and difficult to find. Other solid media include pectin or potatoes.

If You Use Agar: Measure out 1 cup of broth into a saucepan and sprinkle on about 1 teaspoon of powdered or about 1 tablespoon of flaked agar. Let the mixture soak for a few minutes, covered, then boil for about 4 to 5 minutes. Pour the hot mixture

about $^1/_4$ to $^1/_2$ inch deep into the boiled baby food jars and cover with the lids. Refrigerate until use.

If You Use Gelatin: Cool $^1/_2$ cup of broth in your boiled pint mixing jar. Sprinkle one packet (1 tablespoon) of gelatin on the broth and let it soften and partly dissolve for a few minutes, covered. Meanwhile bring the rest of the broth back to a boil. Measure out $^1/_2$ cup of the boiling broth and add it to the gelatin mix to make a total of 1 cup. Swirl the jar to mix and pour the gelatin into boiled baby food jars $^1/_4$ to $^1/_2$ inch deep. Cover and refrigerate until use.

Other Solid Media: How about pectin? Pectin, the other major culinary solidifier besides gelatin, which is used to make jams and jellies, may seem like a logical choice for bacterial culture. However, pectin requires a lot of sugar to work—so much that it tends to inhibit bacterial growth. That's why jams and jellies tend to keep pretty well. However, if you happened to be making jelly anyway, you could pour some pectin into a small jar and experiment with it, to see what bacteria would grow. Fungi (molds) seem to be more tolerant of high sugar, so you might end up culturing those.

How about potatoes? Good idea and very simple. A cooked or freshly cut raw potato is a ready-made substrate for bacterial growth. That's what Koch first used. Just drop it into your "sterile" jar and moisten it with a little boiled water or boiled nutrient broth.

6. Inoculate your solidified medium. Choose an inoculum, such as a few drops of pond water or a swab from your armpit or the surface of a leaf. If the surface is dry, first moisten the cotton swab with a little boiled water. Spread the invisible microbes lightly on the surface of the medium. Try not to break the surface; if you do, however, you will be able to find out whether your microbes can live in the relative lack of oxygen underneath the surface, or whether some microbes can move around in the dense substrate. A toothpick stabbed under the surface is a good way to deliver microbes to those depths. H. Steven Dashefsky, author of several excellent books on science fairs, suggests having an insect walk across the surface to see what was on its feet. When you are done replace the lid either

loosely or tightly. A loose lid lets in more oxygen and favors different species.

7. Choose incubation times and conditions: sunlight? darkness? warmth, such as near a radiator? room temperature? Think about what variables you might control, and experiment with these. Incubation time can range from one to several days. If you are using a gelatin-based medium, the incubation time should be shorter because many of the bacteria you are likely to culture can dissolve gelatin, turning it into a soupy mess. You need to look at your results before this happens.
8. Examine what grew: after a few days, look for colonies on the medium. Shiny, droplet-like colonies of various sizes, shapes, and colors are probably bacteria. However, some yeasts make similar sorts of colonies. Fuzzy colonies are probably fungi, although small ones could be gram-positive actinomycetes.

If you have many colonies growing in and on each other, try using a more dilute inoculum the next time, or try a more dilute (less encouraging) medium. You might even try adding to the medium a small quantity of something that could inhibit growth, such as a bit of sawdust from pressure-treated wood or some household disinfectant. Try soaking bits of paper towel with a weak solution of liquid disinfectant and placing those on the surface of the agar. You might be rewarded with the sight of a zone of inhibition around the paper towel, where no microbes can grow. You can also try boiling your inoculum to select for just the spore-forming gram-positives that resist boiling. Or you can observe the crowded colonies and try to figure out whether any of them are either inhibiting their neighbors (perhaps by means of antibiotics) or enhancing the growth of their neighbors (perhaps by providing nutritious wastes).

If you have little or no growth, try increasing the concentration of nutrients, or try a different inoculum. However, sparse growth of one particular type of colony can be seen as a successful culture method for a specific microbe.

9. If you have a microscope, use a toothpick to pick up a dab of material from a colony. Place it on a slide in a small drop of water, and add a coverslip. Look at the slide under high power

(e.g., 400×), adjusting the light. You may well see rods or coccoids, two of the most common bacterial shapes. If the cells are large and easy to see, and especially if they seem to be made up of long filaments, you are probably looking at fungi.

Mixed Culture Method
The Winogradsky Column

The most famous mixed culture method is the Winogradsky column. Making a Winogradsky column is an excellent project for home or classroom. With little maintenance, a well-established column can last for years. This is a safe experiment; unless you add a large quantity of rotting meat, you are probably not cultivating pathogens. In a sense, you are setting up a specialized compost heap, which can also be done on a small, selective scale on your windowsill (see chapter 11).

In brief, a large jar is prepared with some crumpled paper towel (as a source of cellulose) in the bottom and topped off with rich black sediment from an estuary or mudflat along with some ambient water. After a few weeks to months of incubation, partly or entirely under a light source, a colorful community of sulfur-loving bacteria should develop. The culturing technique is so integral to understanding sulfur bacteria that the detailed description is provided in chapter 9; variations on the technique for the encouragement of metal-oxidizing bacteria and for cellulose-consuming gliders are described in chapters 7 and 15, respectively.

A basic recipe for a Winogradsky column (although Winogradsky himself would probably not have stated it quite this way) is as follows:

1. Find a large jar, preferably tall and cylindrical.
2. Add moderate amounts of ingredients that you hypothesize could be of "interest" to some bacteria. These might include
   straw
   dry leaves
   bits of bark
   pieces of metal
   horse manure
   bits of insect or lobster shells

hard-boiled egg yolk
salt
Epson salts (a source of sulfate)
something acidic or alkaline

You can try just about anything. Use your imagination—think like a bacterium!

3. Top off with mud or soil along with ambient (or other) water.
4. Decide on incubation conditions:
   covered or dark (or create some of each by masking part of the jar with black paper)
   warm or cool
   tightened lid or lidless
   compensation for evaporation (by adding water) or not
   other parameters—use your imagination
5. Watch for layers of colored bacteria to form over a period of days to weeks. Take samples for microscopy from specific layers, taking care to remove any bits of sand that might damage your coverslip. Plan to keep the column for months or even years. The author has had a Winogradsky column decorating her windowsill for more than twenty years.

Percent Solutions

The instructions above tell you how to make solutions using teaspoons and cup measures. In some cases, perhaps for a science fair project, you might want to be able to describe a solution in more scientific terms, as an "X% solution," or you might be using instructions from another source that tell you to make a certain percent solution. Here are some guidelines for using kitchen utensils to create specific types of solutions.

If your kitchen measuring utensils and scales are calibrated in metric units, then the method for making solutions of a certain percentage weight per volume or volume per volume is easy. For example, 1g sugar in 100ml of water is a 1% solution (weight/volume). One ml vinegar in 99ml water is a 1% solution (volume/volume). Even if you have just a set of measuring spoons and cups calibrated in English system units, you can make solutions with certain percentages of ingredients. Keep in mind the following approximate equivalents:

3t = 1T
2T = 1oz = 30g
8T = ½c
2c = ½L

Let's say you would like a 1% infusion of soil in water. One cup of water = 16T = 48t, so you would add 0.48t soil. Round that off to half a teaspoon, unless you have more precise ways to weigh and measure. Half a teaspoon of an ingredient in 1 cup of liquid, then, yields an approximately 1% solution.

Appendix B

Safety Precautions

The caution that one should not drink or eat one's bacterial cultures normally would go without saying. However, several sections of this book elaborate on the roles of bacteria in enhancing the flavors and aromas of food and drink. Therefore, you may be tempted to grow bacteria for the purpose of experimenting with cuisine. Please do not do so unless you are following clear recipes from manuals on cheese making, wine making, pickle making, or the like. This field guide is not intended to provide instructions on how to prepare microbially enhanced foods and drinks.

The tone of this field guide may suggest to some readers that nearly all bacteria are harmless. They are not. Many—especially proteobacteria and gram-positives—are opportunists that can become pathogenic if they gain access to the nutrient-rich conditions of your body. If you choose to culture bacteria, take care not to breathe them in or get them on your skin. Use culture methods as described in appendix A, which are meant to discourage opportunists by using relatively dilute nutrients. This approach better approximates most natural conditions and gives you a better idea of the activities of indigenous bacteria. Using dilute nutrients discourages opportunists, although it does not eliminate them completely. An example of an opportunist is *Staphylococcus*. This species is indigenous to our body surfaces and cavities; however, a *Staphylococcus* infection in which the bacteria have gained access to internal organs, especially if the immune system is not functioning properly, can be fatal.

Take these precautions:

1. Do not eat or drink near your bacterial cultures. This means that while you can prepare bacterial culture medium in your

kitchen and store unused medium in your refrigerator, you should keep your living cultures elsewhere.
2. Keep the lids on your cultures, or remove the lids only briefly as needed. Do not take a deep breath close to uncovered cultures.
3. Wash your hands well after working with bacterial cultures.
4. Wash with rubbing alcohol whatever work surface you are using before and after working with bacteria.
5. When you are done with your cultures, pour in a little bleach. That should kill everything so the cultures can be safely tossed out.
6. While it is common practice in high school and college labs to use rich nutrient agar to culture bacteria, use more dilute nutrients at home. The main reason that nutrient agar is so commonly used in class and science fair projects is that it yields quick and easy results: lots of hardy opportunists. Much more interesting, however, are the slower growing specialists that are normally active in a particular environment. Such bacteria are much less likely to have pathogenic capabilities.
7. Use a "Russian style" mixed culture method (described in appendix A), which does not provide as favorable conditions for pathogenic opportunists.
8. If you have a compromised immune system, your doctor has probably instructed you about what precautions to take. You should not culture any bacteria at all without the permission of your doctor.
9. If you are doing a classroom or science fair project, you are required to follow the guidelines issued by Science Service, Inc., 1719 N. Street N.W., Washington, D.C., 20036; phone: 202-785-2255. If you are working at home on a science fair or classroom project, those same guidelines are applicable. It is suggested that you request a complete set of the guidelines.

Appendix C

How to Use a Microscope

> I beheld with wonder many little animals of divers kinds, which escape our naked eye.
>
> ANTON VAN LEEUWENHOEK, 17th-century Dutch microscopist, as quoted by C. Dobell

To view most bacteria, you need a compound microscope with at least one high-powered objective lens in the range of 40× (40 times magnification). The label "40×" is usually engraved on the side of the lens. If the eyepiece of the microscope is labeled 10× (10 times magnification), then the combined power of eye piece and objective is 40 × 10 or 400×. Even better is a microscope that also has a 100× objective, giving a combined power of 1,000×. Such microscopes are not likely to be found in toy stores, popular science stores, or museum shops, however. The objectives on less expensive scopes typically give you combined magnifications of 200× to 250× or less, sometimes much less.

Where can you find a microscope with a magnification of 400× to 1,000×? You may be able to get access to one from a local high school or college, or sometimes schools and universities that are buying new microscopes are willing to sell their old ones. Used microscopes are also sold at flea markets or through classified ads. It could be worth buying an older scope with the right objective lenses if you want to do a lot of microscopy at home.

Lighting is another factor to consider in choosing a microscope. If you find an old, sturdy scope with no plug-in light but just a little mirror positioned to catch the light, consider it, especially if it has the right lenses. Otherwise, there should be an electric light source. Check to see that it works, keeping in mind that frayed cords and

loose connections can be fixed easily. If you are cleaning up an old scope, use only lens paper to clean the lenses.

Testing the Parts

So now you have a microscope. Or you have borrowed one. Or perhaps you are at a flea market and have somehow found an electric outlet and are trying out a microscope you might buy. Take a close look and try to identify and test the various moving parts.

FOCUSING KNOBS

The microscope should have 1 to 3 pairs of knobs. Check to see how they work. One pair of knobs (perhaps the only pair or the most conspicuous pair) raises and lowers the stage. These are the focus knobs. There may be a smaller set of knobs inside the focus knobs, that do the same thing but in much smaller increments. These are fine focus knobs. As you try the focus knobs, take care that you do not cause the stage to collide into an objective lens.

If there is a second pair of knobs, they are likely to be used for focusing the light. You should find that they move a small lens (condenser lens) system beneath the stage. In general, you should adjust the condenser lens so that it is in the highest position.

MICROSCOPE WITH A MECHANICAL STAGE

If there is a third set of knobs, both situated to one side of the scope, they may move the stage from side to side and forward and back. This arrangement means you have a mechanical stage, which is a nice option on a microscope, enabling you to scan a slide easily.

PHASE-CONTRAST MICROSCOPE

If you have a moveable condenser lens beneath the stage and it seems a good deal more complex than the one described here—specifically, if it seems to consist of a turntable of different lenses—you may have a phase-contrast scope. The word "phase" may appear on some of the objective lenses. These are useful scopes to have in a microbiology lab; however, they are complex enough that a general set of instructions will not adequately explain how to get the lighting right to view bacteria. If you are using a phase-contrast scope at a high school or college, get help from a teacher or professor on the correct use of the condenser. If you have a phase-contrast scope and

no one to assist you, turn the condenser wheel until it is in a position in which you have an unobstructed field of vision. Use it at that setting until you can read the manual or get assistance.

Viewing a Newsprint "Specimen"

Now you have played with the knobs. Next, prepare a specimen for viewing. Do not commence by looking at bacteria if you are a beginner. Newsprint is a good subject for your first observations. Have available a bit of newspaper with some printed letters, a glass slide, at least one thin glass coverslip, and some water. Make a "wet mount" by placing a tiny scrap of newspaper on a glass slide, adding a small drop of water, and placing a coverslip on top. Try to always prepare your slide using a coverslip. Flat preparations, as these are called, are easier to view and pose less risk of getting an objective lens wet. You can buy slides and coverslips at a popular science store, or you may be able to get a few from a biology teacher. If you are careful, you can use them over and over.

Clamp the prepared slide onto the stage using the clips or the slightly more complicated mechanical stage setup, if you have one. Always start with the lowest power (the shortest objective lens), even when your goal is to observe tiny bacteria. Look through the eyepiece and begin to use the focus knob. Newspaper print is so large and distinctive that you should be able to get some letters into focus right away. Note that they are upside down and backward. Gently move the slide from side to side and back and forth to get a feel for the effects of the reversed image. The lowercase letter *e* is an especially useful example.

ADJUSTING THE LIGHT LEVEL

It is important that you become comfortable with light adjustments, as these are critical for observing bacteria. How do you adjust the light? This depends on how complicated your scope is. Here are some of the possibilities:

1. If you have a mirror to reflect light from some other source, try tilting the mirror and adjusting the distance and angle of the light source, which might be an ordinary table lamp. In some configurations, the image will be dark; in others, the light

will glare too brilliantly. Find an optimal middle point. One advantage of this setup is that you can use your scope outdoors in sunlight.

2. If you have a built-in light bulb with an on/off or dimmer switch, use that to adjust the amount of light reaching the stage.
3. Whether you are using a light bulb or a mirror, there may be a lever that opens and closes a diaphragm beneath the stage. Use it to optimize the light level.

If all looks fine at the lowest power, rotate the next highest power lens (the next longest) into place. You should find that the newsprint is almost in focus with this lens and that you need to do only a little adjusting, usually just with the fine focus. However, you may need to adjust the light. Remember that adjusting the light does not usually entail moving the condenser up and down. Keep that in the top position. Use all of your objective lenses in turn up to the 40×. Pause there before using the 100× (if you have one).

USING THE OIL (100×) OBJECTIVE

The 100× objective is the most powerful (and most expensive) lens on any microscope. It can be used only with immersion oil—never without. The word "oil" may appear printed on the side of the lens. Using the lens without oil could scratch it. Immersion oil has been formulated to be free of solvents, acids, and other contaminants that might harm your scope. You should be able to obtain immersion oil from a high school or college. If not, resist using your 100× lens until you are able to obtain the oil from another source.

Here is how you use the oil lens:

1. Prepare a wet mount, taking care that it is not too wet or too thick.
2. Use a toothpick to transfer a tiny drop of oil to the top of the coverslip.
3. Peering at the oil lens from the side, gently lower it (or raise the stage) until the lens just touches the oil, causing the oil to spread a little.
4. Look through the eyepiece and *very gently* focus up and down, until an image is in focus. Remember to adjust the lighting too.

What can go wrong with your oil lens? You can crack it by focusing it too vigorously so that it collides with the slide. You can clean off the oil with a tissue or other rough paper, scratching the lens. Use only lens paper, and consider letting a little oil remain on the lens if you are planning to use it again soon. Try not to get the oil on the other lenses or on the stage.

VIEWING REAL SPECIMENS

After your practice explorations using newsprint, get ready to look at real field samples that include bacteria as well as other microorganisms. Murky pond water is a classic choice. Collect some in a jar, including a few leaves and some sediment, and set it on a windowsill. Over the course of a few days, a microbial community will develop. Take a small drop plus a little scraping from a leaf surface, and make a wet mount. Start with low power, adjusting the light. If you do not immediately find microbes, focus on some debris.

At the lowest power, whatever organisms you see are likely not to be bacteria but rather some sort of protists (e.g., a ciliate) or small animal (e.g., a rotifer or a nematode). They are fascinating in their own right and deserve hours of observation. However, the goal of this appendix is to get you to see bacteria. Adjust the magnification incrementally up to 40×, stopping at each lens to focus and adjust the light.

At 40× (a combined magnification of 400× with your 10× eye piece), you have the possibility of seeing bacteria. Keep in mind that most bacteria are tiny colorless rods and coccoids (dashes and dots) that will not be moving much on your slide. There are exceptions—large, colorful, active bacteria—but those are found only in specific environments, as mentioned throughout this field guide.

Right now, however, you are probably looking at a sample that is full of rather ordinary dashes and dots, 10 to 100 times smaller than the protists. Dimming the light a bit might make them seem a little less transparent. Also, try allowing the slide to sit for several minutes before you view it. This gives the bacteria a chance to make contact with the slide and makes them easier to see.

Frustrated? Pond water is a great introduction to the microbial world and is a good way to start using your microscope on real field

samples. However, the bacteria in your sample may prove disappointing or frustrating, as they sometimes are even to professional microbiologists.

You might want to try another sample. Take some beef or chicken broth, noncreamy style with as little fat as possible. Set it out in an open container on your counter for 24 hours or more. Make a wet mount of a drop of broth, and view it with each of your objectives, lowest to highest. At the low powers, you may see nothing, but focus anyway on a bubble or bit of debris. At 40×, you may find your sample teeming with bacteria, including rather long rods and active motile forms. Enjoy! Your soup sample is thoroughly contaminated with opportunistic bacteria that were dormant in your kitchen until you provided this feast.

TIPS FOR VIEWING FIELD SAMPLES

At this point, you are ready to follow the instructions given throughout this guide for collecting and observing particular samples, in the sections titled "Viewing under a Microscope." One admonition, repeated frequently, is to avoid making preparations that contain any sand whatsoever. You must either pick out the bits of sand, grain by grain, or dilute your sample away from the sand. Otherwise, you will crack your coverslip trying to make a flat mount. Also, keep in mind that less is more with bacterial preps; do not use large, overflowing drops. Always begin your focus sequence at the lowest power, even if it means focusing on debris. If you cannot focus at low power, you will have even greater difficulty at higher powers. Adjust the light, taking into consideration that your subjects are often transparent. Also, most preparations benefit from being allowed to settle for several minutes so that the bacteria can make contact with the glass slide. Having most of your bacteria in the same plane of focus, against the slide, makes them easier to see.

The combination of microscopy with the culture methods described in appendix A and throughout the book can be powerful. A little dab of a cultured bacterial colony or film or scum, taken up on a toothpick and prepared with a small drop of water, will likely show you hordes of tiny cells, perhaps with one particular type predominating.

Appendix D

Suggestions for Science Fair and Classroom Projects

Consider a different route from that taken by so many science fair participants: don't test mouthwash or soaps or any of the household or personal cleaning products by culturing mouth and skin bacteria before and after. That is, don't do this unless it truly excites and intrigues you and you are willing to ask additional questions and make it more of an exploration. I write this as a judge of science fairs. I've sometimes found that nearly all of the microbiology projects at a science fair are some variation of a before and after testing of household products.

Instead, be more adventurous. After all, you have *A Field Guide to Bacteria* in your hands, which is in itself an unconventional approach to bacteria. Skim through and choose a bacterial group because it seems interesting. Then read the whole chapter for that group, taking notes as you go. What questions do you have that seem to be unanswered, at least by this book? Try to come up with several. Later, when you are considering experiments, some questions may be answered with further reading, whereas others might be too complex for a short-term project. Settle on what seems to be a good question, such as "What conditions are favored by the big, white, conspicuous bacterium *Beggiatoa*?" You know from reading chapter 9 that it likes a certain amount of hydrogen sulfide and oxygen. How quickly can it move to a new position to be in the right gradient of these chemicals? The question could be approached both by experiments in the field and by designing Winogradsky columns with varying conditions. Where do you find *Beggiatoa*? That's why you have this field guide.