Maintenance of Bacterial Strains

Short-term growth and maintenance

The simplest method for obtaining strains is to order them from a microbiological supply company. Presque Isle, Ward, and Carolina Biological (see "Resources" page of the Microbes Web site: www.umsl.edu/~microbes) are economical sources of strains that are commonly used in the classroom. Another possible source is a local college or university that teaches a microbiology lab course. If they have strains already prepared for their class, they will probably be willing to provide you with a culture. This is probably not a reasonable ongoing source of strains, but can be useful in an emergency.

For short-term maintenance and use, it is best to streak bacteria on some type of rich agar petri plate, such as nutrient agar. Label and date the plate on the bottom of the plate. Streak from the source culture using good sterile technique. The quadrant streak method (see the article on quadrant streaking) is good for obtaining well-isolated colonies. Incubate the plate only until good colonies have formed. Do not leave the culture in the incubator beyond that time, or the cells will die on the plate. Store the plate in the refrigerator. It will keep for about a month. For class use, it is best to streak a fresh plate 2-3 days before the strain will be needed for class. Use the fresh plate as the source of cultures for the class.

For broth cultures for a class, it is best to grow a larger volume culture in a flask (if possible, with shaking) overnight and then dispense aliquots of the master culture into sterile test tubes using a sterile pipette. Once broth cultures have been used by students they should be disinfected and discarded, since they may have been contaminated during use.

Agar plates are the preferred medium for short-term storage of bacteria because it is easy to verify that the plate contains only one type of colony, or to see contaminant colonies if they arise. The most frequent contaminants are strains of *Bacillus* sp. These often produce spreading, dull, milky or chalky-opaque colonies with an irregular shape. If a plate is very contaminated it should be discarded; however, if there is an area of the plate with only the desired colonies, one of these colonies can be carefully picked with a sterile transfer loop and streaked to a fresh plate of agar. Any streak plate should be carefully examined to be sure that there is only one colony type on the plate. If there are older streak plates, it is useful to compare the colonies on the two plates to be sure that they appear similar. If there is more than one type of colony and it is not evident which is the correct one, each type must be streaked separately on an agar plate and tested to determine which is the correct strain. (If there is time, it may be easier to order a new strain.)

Agar slants contain the same medium as petri plates, but in a tube in which the agar has solidified while the tube is on a slanted surface. A slant has the advantage of a screw-cap top that prevents the agar from drying out; however, it is difficult to determine whether a culture growing on an agar slant is contaminated.

Preparation of Slant Cultures

1. Place screw cap test tubes in a test tube rack (without the caps).
2. Prepare a nutrient agar medium and boil it with stirring until all the agar is melted. You must stir this very well so that the melted agar is distributed throughout the medium.
3. Use a pipette to transfer about 5 ml of molten agar to each test tube.
4. When all the tubes contain hot agar, place the caps loosely on the tubes and sterilize the tubes.
5. While the medium is till hot, tilt the rack onto a thick book or other solid surface so that the medium in the tubes is slanted. Allow the medium to harden in this position.
6. When the medium is cool, tighten the caps.
7. To inoculate a slant, use an inoculating loop to transfer cells from a single colony on a plate to the surface of the slant. Move the loop back and forth across the surface of the slant. Cap the tube, and incubate until growth is evident, then refrigerate the tube.

Long-term maintenance of strains

Long-term maintenance of bacteria is not easy in the typical classroom, and is not generally recommended. While lyophilization is the best method for long-term storage, it required a lyophilizer, which is not usually available. Another method uses very low temperature (about –70°C) storage of cultures that include a cryoprotectant such as dimethylsulfoxide (DMSO) or glycerol. Some strains can be stored for 1-2 years in agar stabs, which are similar to slants except that the cells are inoculated deep into the agar and the tubes are sealed with wax to exclude air. While the last method is not difficult, it is not clear how many different strains survive well in the stab.

Since frozen stocks of most bacteria survive well, this is probably the best method for the classroom. Although most instructors do not have access to an ultracold freezer, even a freezer at -20°C should preserve most strains for months/years. You will need a small bottle of dimethylsulfoxide (DMSO) (to be used only for this purpose, so that it does not become contaminated), some small sterile tubes or vials, a holder for the tubes, and space in a -20°C freezer.

<u>To store a strain in the freezer:</u>

1. Autoclave small (1-3 ml) screw-cap or snap top tube s (microcentrifuge tubes are fine).
2. Grow a fresh overnight culture of the strain in broth. Do not grow the cultures too long.
3. Label the sterile tube with the strain and the date.
4. Use a sterile pipette to transfer 0.2 ml of DMSO to the labeled tube.
5. Use a second sterile pipette to transfer 1.0 ml of broth culture to the tube with DMSO.
6. Invert the tube several times to mix.
7. Place the tube in the -20°C freezer.

<u>To recover a strain from the freezer:</u>

1. Label the bottom of a petri plate containing an appropriate medium with the name of the strain and the date.
2. Carry the plate and a beaker holding several sterile toothpicks to the freezer.
3. Remove the tube containing the strain of interest.
4. Remove the cap.
5. Quickly scrape a small amount of the frozen cells onto the toothpick.

6. Transfer the material to the agar surface of the plate.
7. Quickly replace the cap and put the tube back in the freezer before it thaws.
8. Take the plate back to the lab and use a sterile inoculating loop to streak from the melted material to make a typical streak plate.
9. Colonies should appear in 1-2 days.

If no colonies appear, thaw the entire tube and transfer the contents to a flask of broth. If the broth shows growth in 1-2 days, streak a plate from the broth and verify that it is the correct strain. Remake the frozen stock from a new broth culture started from a single colony on the streak plate.

Tips for success in growing and maintaining strains

- As soon as you receive a culture streak it on an agar plate. Refrigerate the plate as soon as it grows well.
- Plates and slants keep better than broth cultures.
- Start seed cultures from a single colony.
- Grow strains in an appropriate growth medium near the optimum temperature.
- Grow strains that require oxygen or prefer to have oxygen in flasks with a large surface:volume ratio and shake vigorously if possible.
- If you want to maintain cultures in broth, subculture broth cultures once a week.
- Grow cultures only until there is good visible growth and then refrigerate the culture to maintain cell viability. Do not store cultures in the incubator.
- Store all cultures in the refrigerator.
- Just because you see cell growth on a plate or in a broth culture does not mean the culture is alive. Dead cells generally look the same as live cells.
- If a strain appears to be contaminated (more than one colony type on a streak plate) order a new culture.
- Maintain strains on streak plates and check carefully for contaminants.
- Restreak cultures on slants or plates about once a month.

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