

Why Won't My Cultures Grow?

A common problem for microbiology activities in the classroom is that the culture needed for the experiment fails to grow. For a complete discussion of bacterial growth that will help you to understand why these problems occur, please see the article: [Introduction to Bacteria](#).

The sections below include a short description of some common problems and solutions to those problems.

- Problem:** • A fresh plate or broth culture inoculated from a plate culture, slant culture or broth culture shows no growth after 24 hours.
- Reason:** • The most common reason for this problem is that the source culture was dead. Dead bacteria generally look the same as live bacteria, so you cannot assume that cells on an agar surface or in broth are alive.
- Another possible problem is that you did not provide the correct medium or growth conditions for the strain.
- Solution:** • Transfer broth cultures once a week.
- Transfer plate or slant cultures once a month.
- Grow cultures only until there is healthy growth, usually overnight.
- Do not keep plates over a weekend in an incubator.
- Refrigerate cultures after there is growth. Do not let them grow too long.
- If the culture used as an inoculum was young, see the description of the next problem for possible solutions.

- Problem:** • The culture is growing, but very slowly.
- Reason:** • Bacteria usually grow rapidly when they are provided with optimum growth condition, but slowly when their needs are not met.
- They need sufficient nutrients, the proper pH and salt concentration and the right temperature.
- Fungi (except Baker's yeast) typically grow slowly, so expect them to take 3-5 days to grow well.
- Solution:** • Determine the optimum conditions for the strain and provide it as best you can.
- Most bacteria prefer to grow aerobically, so growing cultures in flasks with a large surface area:volume ratio will help to provide oxygen.
- Shaking is desirable, but not usually necessary.
- Most bacteria used in the classroom grow well in nutrient broth or nutrient agar.
- Yeast grow better in a growth medium that contains sugar. Baker's yeast grows fine in sugar and water.
- Most bacteria and fungi grow slowly at room temperature. An incubator can help to speed up growth.

- Problem:** • There were bacterial cells on the plate or in the broth but they disappeared.
- Reason:** • Some strains, notably those of the genus *Bacillus*, destroy their cells when they die. In this case you can tell when a culture is dead because the cells lyse and are no longer visible.
- Solution:** • If the problem occurs with a *Bacillus* strain, you may be able to rescue the

culture by transferring some of the invisible cell material to fresh broth. Many strains of *Bacillus* produce resistant spores before the vegetative cells lyse and it requires only a few (invisible) spores to start a new culture.

- If you cannot rescue the strain you will have to obtain a fresh culture.

Problem: • The culture plate has colonies that do not look like most of the colonies, or there are colonies where nothing was streaked.

Reason: • The plate has become contaminated with bacteria or fungi from the environment.

Solution: • If there is little contamination and it is clear which colony type is the desired one, restreak from a single colony.

- Use a sterile inoculating loop or a sterile toothpick to pick a single, well-isolated colony of the desired type and restreak that single colony on a fresh plate.
- Check it carefully after it grows to be sure it is the correct strain and that it is pure (free of any contaminants).

• Alternatively, obtain a fresh strain.

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