

## Temperature Effects on Growth Lab

Most bacteria grow within a particular temperature range (Figure 1). The **minimum** growth temperature is the lowest temperature at which a particular bacterium can still grow, while the **maximum** growth temperature is the highest temperature at which a bacterium can still grow. The **optimum** growth temperature, however, is the temperature at which the bacterium grows at its highest rate. When an organism is grown below its optimum and above its minimum temperature, it grows much more slowly because the metabolic enzymes are performing inadequately for maximum growth and the plasma membrane becomes rigid and unable to transport nutrients effectively. Likewise, when an organism is grown above its optimal temperature the metabolic enzymes start to denature and quickly become nonfunctional, which slows the growth and actually kills the organisms when the temperature is too elevated.

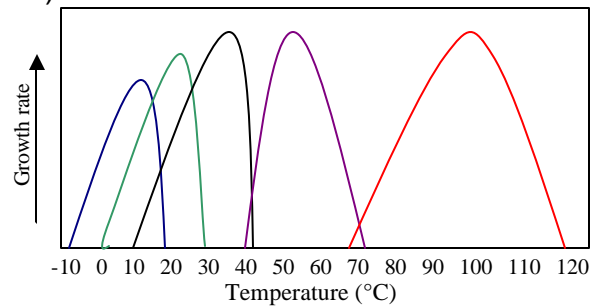
Bacteria can be classified into five different groups based on their growth properties at various temperatures (see figure 1); **psychrophiles, psychrotrophs, mesophiles, thermophiles and hyperthermophiles**. Table 1 and Figure 2 show the temperature ranges for these different bacteria. Thermophiles and hyperthermophiles thrive in hot environments simply because their enzymes have a thermostable structure that makes them resistant to heat, and their plasma membranes have more saturated fatty acids that help the membranes to maintain their integrity in the presence of heat. Psychrophiles and psychrotrophs thrive in cold environments because their membranes have more unsaturated fatty acids which allows them to maintain fluidity, which aids in proper nutrient transport into the cell.

Sometimes bacteria produce pigments when they grow. For instance, *Serratia marcescens* produces a pink pigment called prodigiosin. Since production of pigments takes a lot of energy most bacteria will only produce these pigments when they are growing optimally.

In this lab we are going to investigate the growth of 3 different organisms (*Escherichia coli*, *Serratia marcescens* and *Bacillus sterothermophilus*) grown at 5 different temperatures (4, 25, 37, 42 and 55 degrees C). Before we start, make some predictions (do question #1 on the data sheet).

**Table 1.** Temperature ranges for different bacteria.

Classification	Temperature Range (°C)	Optimum Growth Temperature (°C)
Psychrophile	-10 to 20	10
Psychrotroph	5 to 30	25
Mesophile	10 to 45	37
Thermophile	40 to 75	55
Hyperthermophile	65 to 120	90-100



**Figure 1.** Growth response of bacteria in their growing temperature range.

**Materials per group (Break up into groups of 4.)**

1. 5 nutrient broth tubes
2. 2 nutrient slants

**Cultures per group**

1. SM (*Serratia Marcescens*)
2. See table 2 to determine which second culture you will be using. In the protocol, whenever you see “**second culture**” it is referring to this culture.

Table 2. Group Second Culture

Group #	Culture
1, 2, 3	EC
4, 5	BSt
6, 7	SM

**Protocol**

**First Lab Period**

1. Label each of your slants with the date, your group number, and SM. Label one slant 25 (to represent a 25 degrees C incubation) and label the other slant 37 (for 37 degree C incubation).
2. Inoculate each slant with SM and place in appropriate incubator.
3. Label your five broths with your group number, date and the initials of your second culture. Label one tube 4, another 25, another 37, another 42 and the last one 55.
4. Inoculate each of the 5 broths with your second culture and place each of them in the appropriate incubator (the incubator that matches the label).

**Second Lab Period**

1. Obtain your tubes from the respective incubators.
2. Swirl each broth to mix and examine the growth.
3. Fill in the data on your data sheet for the level of growth in your broths. Put ++ for lots of growth, + for some growth and – for no growth. If any of the broths produced a pinkish color that means it grew very well (the darker the pink the better the growth).
4. Now fill in the data on your data sheet for the level of growth in your slants. The darker the pink and the more dense the growth on the slant, the better the growth.
5. Answer the questions on the data sheet.

**Data Sheet for Temperature Effects Lab**

1. Predict the level of growth for each of the different types of organisms when they are grown at the indicated temperature. Put a ++ for most growth, + for some growth and a – for no growth.

Organism	5 °C	25 °C	37 °C	42 °C	55 °C
Psychrophile					
Psychrotroph					
Mesophile					
Thermophile					
Hyperthermophile					

2. Observe your broth cultures during day 2 of the lab. Record their actual growth as +, ++ or – and indicate what type of organism each species is based on their growth patterns.

Species (EC, SM, or BSt)	5 °C	25 °C	37 °C	42 °C	55 °C

3. Put your data on the board for your group and copy the data from other groups for the species you don't have in the table below. In the column marked classification, determine what type of organism each species is based on their growth patterns (i.e. mesophile, psychrotroph, thermophile, etc.).

Species	5 °C	25 °C	37 °C	42 °C	55 °C	Classification
EC						
SM						
BSt						

4. Describe what the growth of SM looked like in the slant incubated at 25 degrees C and 37 degrees C.
  - a. 25 DEGREES C:
  - b. 37 DEGREES C
5. What is the optimal growth temperature of SM? Give two reasons why you chose that answer.
6. Why doesn't EC grow well at 55 degrees C? What happens to it specifically?