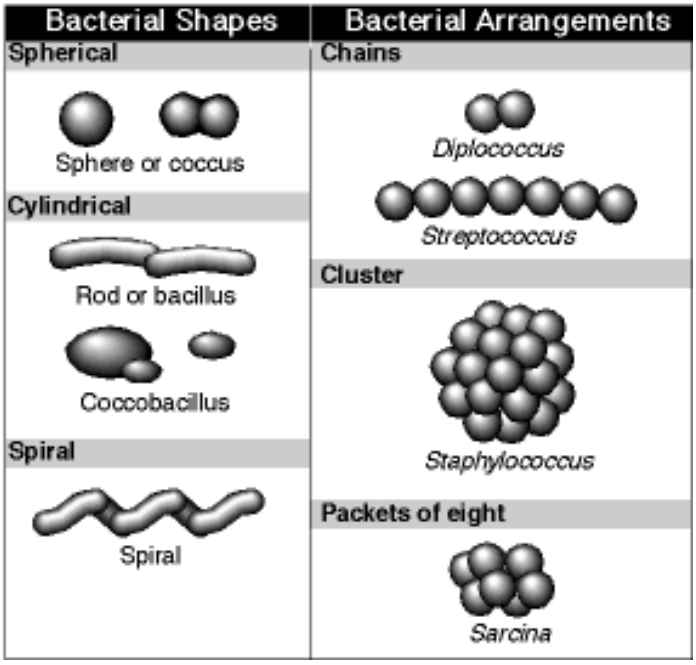
INTRODUCTION TO BACTERIA

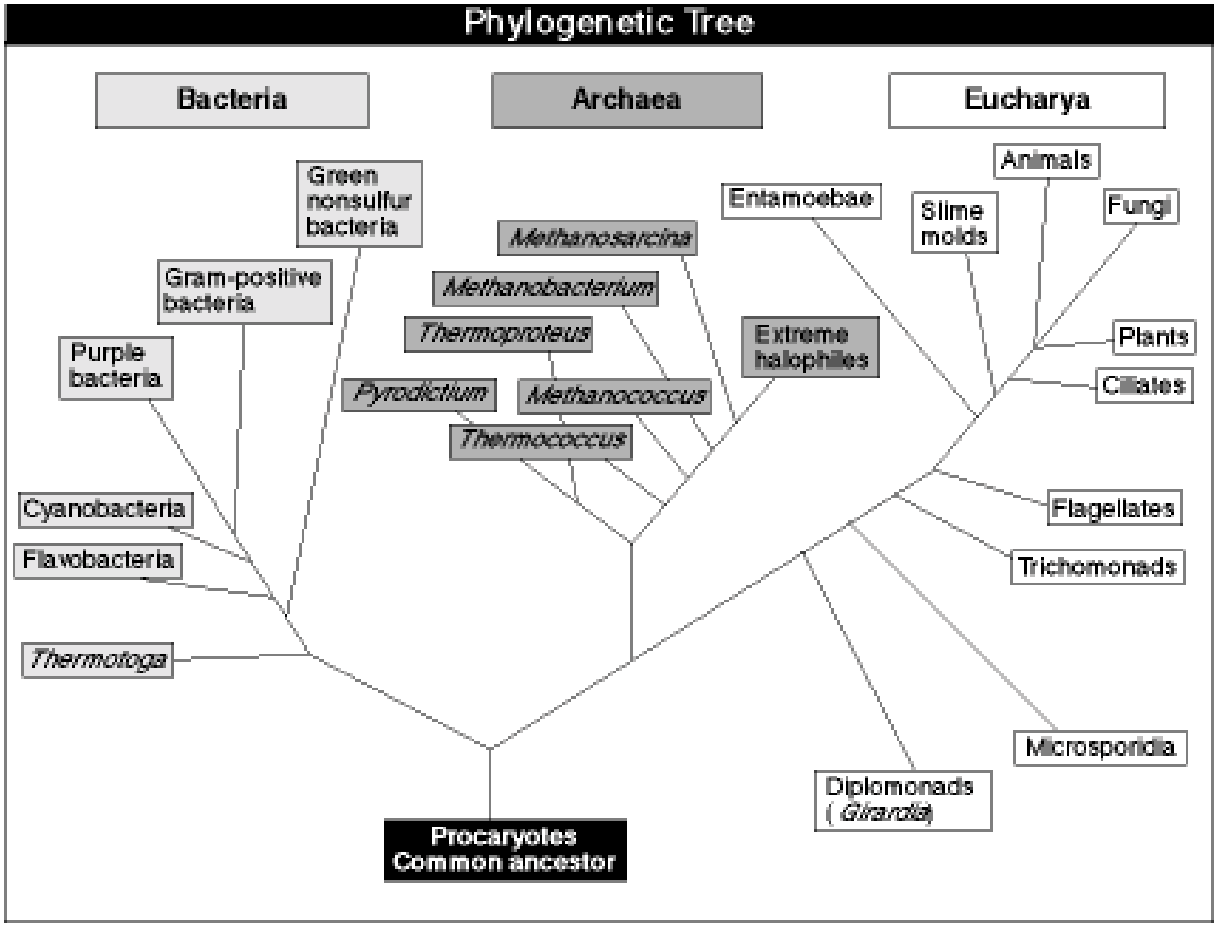
Morphology and Classification

Most bacteria (singular, bacterium) are very small, on the order of a few **micrometers μm** (10^{-6} meters) in length. It would take about 1,000 bacteria, one μm in length, placed end-to-end to equal one millimeter, which is about the width of a pencil line. In fact, however, bacteria come in a wide variety of shapes and sizes, called the **morphology** of the organism. The most common shapes are rod-like, called the **bacillus** (plural, bacilli) form, or spherical, called the **coccus** (plural, cocci) form. The rod forms vary considerable from very short rods that almost look like cocci, to very long filaments thousands of microns in length. Bacteria also form spirals and corkscrews, ovals (coccoid), commas, and elaborately branched structures. The cocci often take on multi-cell forms; as two cocci joined together (diplococci), as chains of cocci (streptococci), or as tetrads (four cells in a cube).



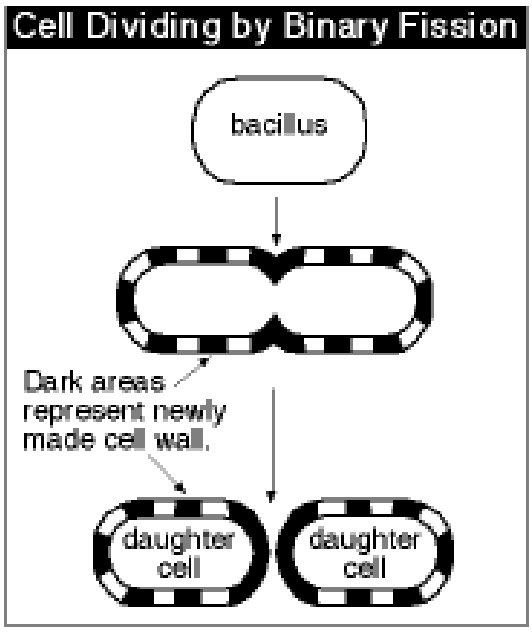
A second major criterion for distinguishing bacteria is based on the cell wall structure. There are types of cells wall that give different staining characteristics with a series of stains and reagents called the **Gram stain**. Bacteria with a thin wall layer and an outer membrane stain red with this protocol and are called **Gram negative**. Bacteria with a thicker wall layer, lacking the outer membrane, stain violet and are called **Gram positive**.

There is a major division of the bacteria that are now classified as a separate kingdom, called the **Archae**. These bacteria different in many important ways from the bacteria that are now called the **Eubacteria**. The Archae include many interesting bacteria with unusual metabolic capabilities such as those that produce ethane. Bacteria that are used in the classroom are usually members of the Eubacteria.

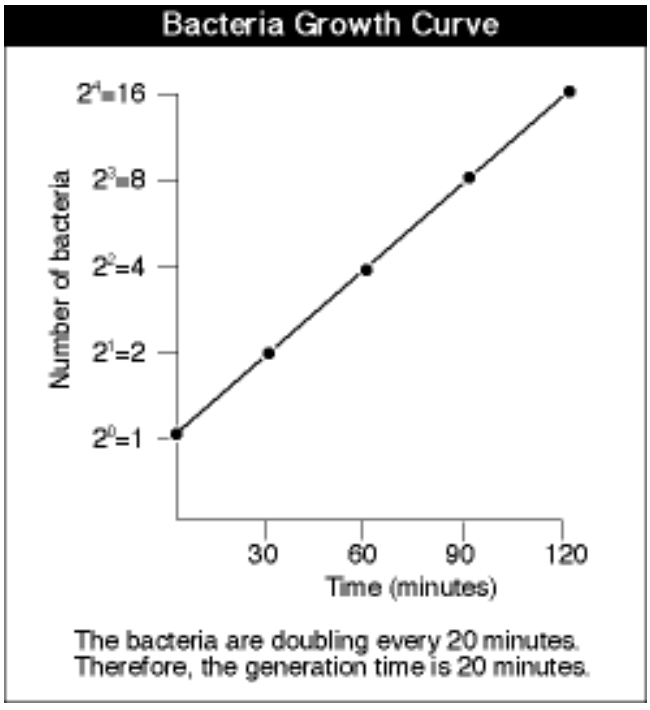


Growth

Although widely varying in morphology, bacteria share one major characteristic: they divide by simple **binary fission**. This means that one cell grows to about double its original size and then splits into two genetically identical cells. Since DNA replication occurs before the cells divide, each new cell, called a daughter cell, gets a complete genome (a full set of genes). The two genetically identical daughter cells are called **clones**. All the progeny of a single original cell form a mass of cells on a solid surface such as agar that is called a **colony**. If the original form was not a single cell, for example, it was a chain of cocci, that entire chain of cells and all its progeny will form a single colony. So a **colony forming unit (CFU)** may include the progeny of a single cell, or it may include the progeny of several cells that were originally connected to each other.



The mathematics of bacterial growth is fairly simple, since each original cell divides to form two new cells, with the loss of the original parent. Since one cell becomes two and as each of those cells divides they become four and then eight, etc., the mathematical series describing growth is: 1, 2, 4, 8, 16, 32, 64, …. This can be written as 2^0, 2^1, 2^2, 2^3, 2^4, …. Therefore, this is a series in base 2. It is an **exponential** series, since the number that increases in the series is the exponent. Thus, bacteria show exponential growth.

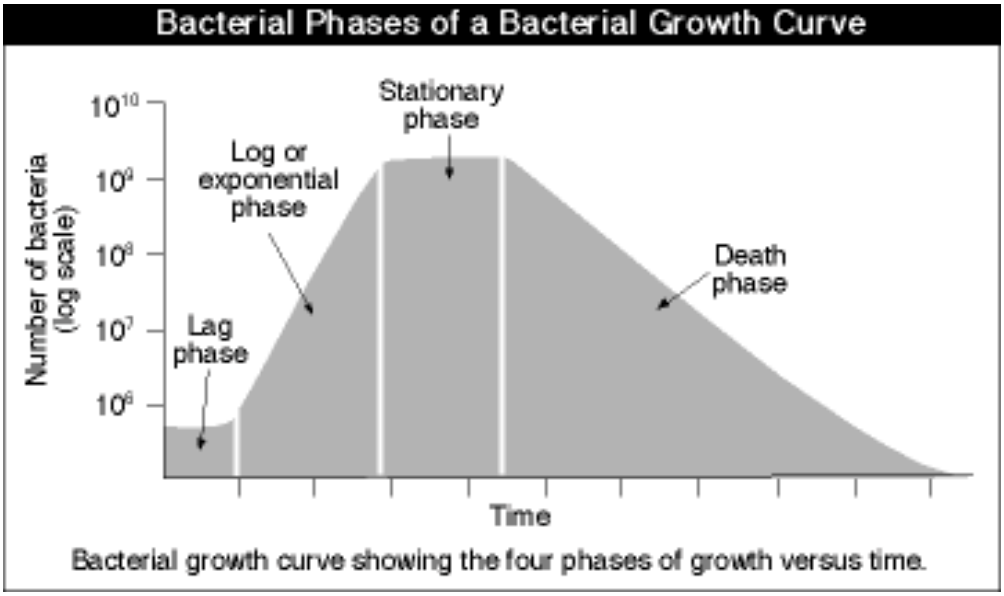


Exponential growth leads to rapidly increasing populations. For example, a bacterium that divides every 30 min has a **generation time** of 30 min. Every 30 minutes the population doubles. In 30 min the population increases two-fold; in one hour, 4-fold; in two hours, 16-fold; in 24 hours it would theoretically increase over a hundred-trillion-fold. In fact bacteria do not grow to such a high population density because their growth becomes limited as the population density increases.

Bacterial Growth Curve

Bacterial growth over time can be graphed as cell number versus time. This is called a **growth curve**. The cell number is plotted as the log of the cell number, since it is an exponential function. Regardless of the generation time, in a growing culture the plot of the log of cell number versus time gives a characteristic curve. This curve typically has four distinct phases: lag phase, exponential (log) phase, stationary phase, and death phase.

In cells that have been freshly inoculated into a new growth medium, the **lag phase** is the first phase observed. It is characterized by no increase in cell number; however, the cells are actively metabolizing, in preparation for cell division. Depending on the growth medium, the lag phase may be short or very long. For example, if a culture in a rich growth medium that supplies most of the cells' requirements is inoculated into a poor medium that requires the cells to make most of their own amino acids and vitamins, the lag phase will be very long. The cells must activate the metabolic pathways for amino acid and vitamin synthesis and must make enough of these nutrients to begin active growth. In contrast, cells that are simply diluted from one medium to a fresh tube of the same medium may show virtually no lag phase, since they need not change their metabolism.



Bacterial growth curve showing the four phases of growth versus time.

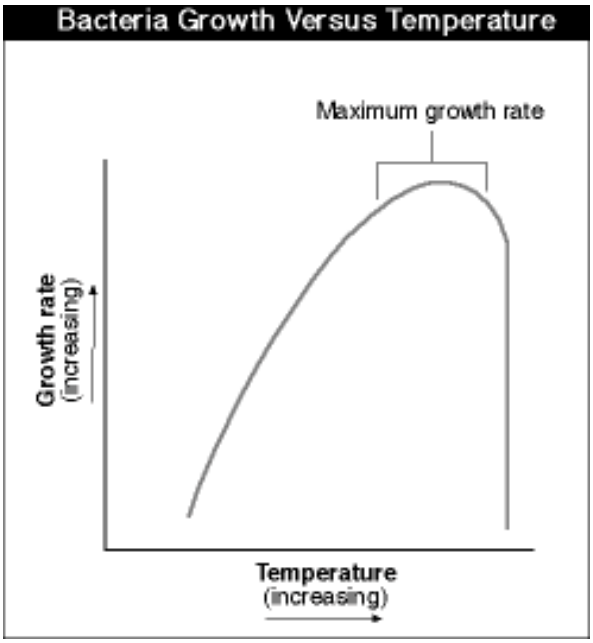
Once cells are actively metabolizing they begin DNA replication and shortly after that the cells divide. This begins the second phase of growth called the **exponential or log phase** of growth. This is the period in which the cells grow most rapidly, doubling at a fairly constant rate. The time it takes the culture to double is called the **generation time**. The generation time can be easily obtained from the exponential phase of a growth curve. The log of the cell number versus time will yield a straight line when the cells are in exponential growth. The generation time can be read directly from the graph using two points on the straight line that represent a two-fold increase in the cell number.

The generation time depends on several factors: the organism itself, the growth medium, and the temperature are all important factors in determining the generation time. Under constant conditions, the generation time for any organism is quite reproducible, but differs greatly among different bacteria. The fastest growing bacteria have generation times of 15-20 min under optimum growth conditions. Many bacteria, however, have generation times of hours or even days.

The third phase in the growth of bacteria is **stationary phase**, when metabolism slows and the cells cease rapid cell division. They may divide slowly for a time, but soon stop dividing completely. They are still alive and maintain a slow metabolic activity. The factors that cause cells to enter stationary phase are related to changes in the environment, typically caused by high cell density. Among the changes that slow growth are depletion of nutrients and accumulation of waste products. If cells in stationary phase are diluted into fresh medium they quickly resume exponential growth.

The final phase of the growth cycle is the **death phase**. In this phase the cells quickly lose the ability to divide even if they are placed in fresh medium. Like the phase of rapid growth, the death phase is also exponential; therefore, cells die quickly and within hours a culture may have no living cells. The death phase, and in fact all the phases, can be slowed by lowering the temperature. Hence, **in order to maintain maximum cell viability it is best to grow bacterial cultures only to early stationary phase and then chill the culture.** Leaving a culture at the optimum temperature for growth for a long period of time simply accelerates the death of the culture. Most dead bacterial cells look identical to live cells, so the normal appearance of a liquid culture or of colonies on a plate is no indication that the cells are alive.

Factors that Affect Growth

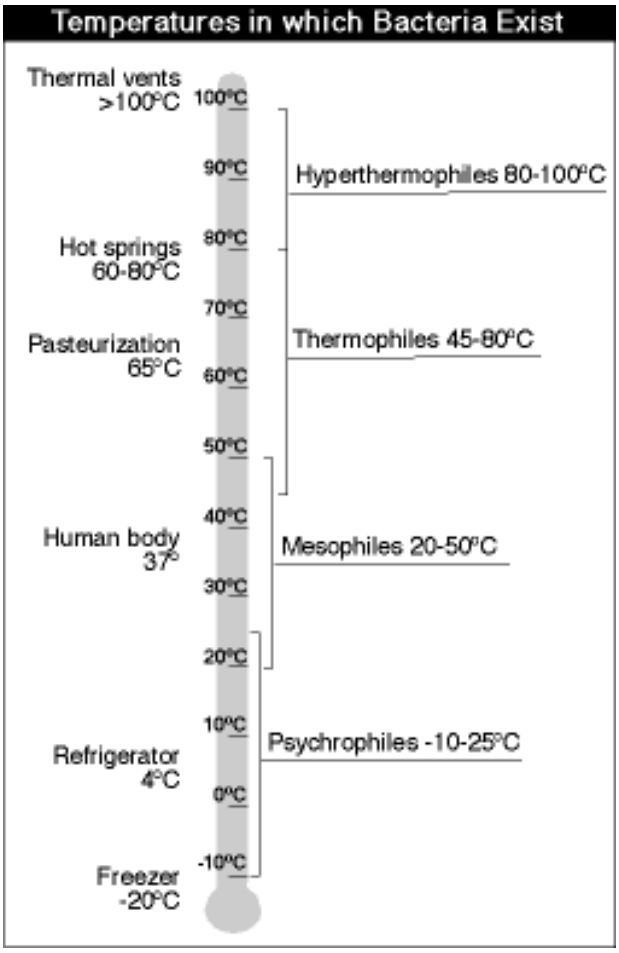


Many factors affect the generation time of the organism: temperature, pH, oxygen, salt concentration and nutrients are some of the more common factors that may change in the normal environment of bacteria. While most bacteria grow best when these parameters are optimum for that strain, in the real world microbes can expect frequent environmental changes. In fact some bacteria have evolved to thrive in environments that are inhospitable for most life.

The temperature in many natural environments changes drastically over the seasons. While most of the well-characterized bacteria live best at temperatures from 25°-40°C, many bacteria thrive at high temperatures and others

grow best (although slowly) at 0°-15° C. Every organism has an optimum temperature for growth; the generation time increases as the temperature declines from that optimum. As the temperature increases beyond the optimum temperature, the generation time also increases until it drops to 0 when the heat kills the cells. Temperature control is one of the major methods for preserving food from the deleterious effects of microorganisms. Very high temperatures kill microbes. Very low temperatures and moderately high temperatures increase the generation time, thus slowing growth to preserve the food.

Those bacteria that grow best at ambient temperatures are called **mesophiles**, while those that have an optimum temperature above about 45°C are called **thermophiles**. The bacteria that grow best from about at 0°-15° C are called **psychrophiles**. Although psychrophiles grow best at low temperatures, they grow very slowly. Thermophiles grow poorly at ambient temperatures, preferring very hot environments; however, there is wide variation in thermophiles. Some that grow well at 100°-120°C are now called **extremophiles**. These bacteria can be found in thermal vents at the bottom of the ocean where high-pressure vents produce temperatures well above boiling.

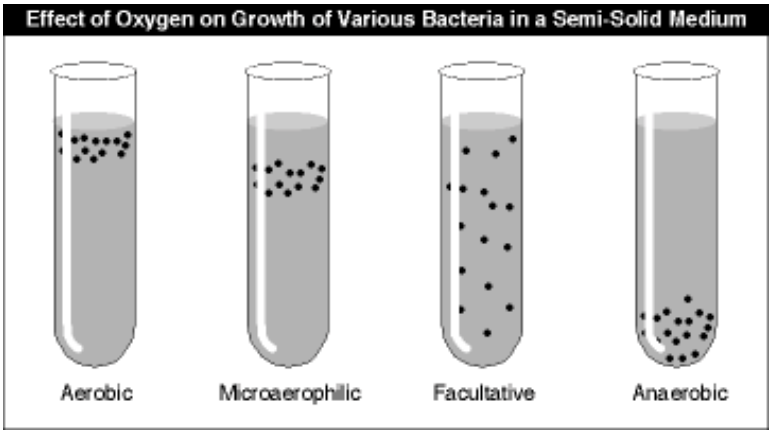


There is great interest in studying and using the enzymes from extremophiles that can withstand very high temperatures. One example of such an enzyme is the DNA polymerase used in the polymerase chain reaction (PCR). PCR requires high heat to separate the strands of DNA; however, high temperatures inactivate the DNA polymerase from most organisms. The bacterium *Thermus aquaticus* was one of the first sources of a heat-stable DNA polymerase, called *Taq* polymerase. This enzyme and several others from thermophilic bacteria have helped to revolutionize molecular biology.

The pH of the environment affects bacterial growth. Most bacteria grow best in the pH range from about 6-8; however, there are many acid-tolerant bacteria as well as alkaline-tolerant strains. In general, bacteria survive alkaline pH better than acid pH, but a few strains actually grow better in an acidic environment. Some can even use sulfuric acid as an energy source. The pH of the cell contents of bacteria that grow in acidic or alkaline environments is neutral. These strains have transport mechanisms to keep a normal physiological H^+ ion concentration inside the cell. Control of pH is also a method of food preservation, used primarily in pickling. The acidic environment of the pickling solution prevents microbial growth.

The salt concentration in an environment is the major contributor to the osmotic effect of ions on growth. Bacteria require ions that are provided by salts and typically tolerate moderate salt concentrations. High salt or high sugar in the environment leads to loss of water from cells and, ultimately, to death. This is the basis for preserving foods using high concentrations of salt or sugar.

We live in a salty world so it is not surprising that may bacteria thrive in high salt environments. These **halophilic** (salt-loving) bacteria are called halophiles. Many halophiles belong to the bacterial Kingdom called the Archae. Halophiles have mechanisms to actively pump out salt, keeping the inside of the cell at a normal salt concentration.



We think of oxygen as essential for life, but oxygen is a reactive and potentially toxic molecule. Many bacteria prefer to grow in the absence of oxygen, and for some strains oxygen is highly toxic. Bacteria are called **aerobes,** if they require oxygen for growth. These bacteria can only make energy from respiration, which requires oxygen. Many bacteria grow with or without oxygen; these are called **facultative aerobes**. They have respiration, but can also grow by fermentation, which produces energy without oxygen. Since these bacteria obtain more energy by respiration than by fermentation, they grow faster with oxygen. A third group, called the **aerotolerant anaerobes**, comprises bacteria that cannot use oxygen because they lack respiration, but are not killed by oxygen. They generally prefer environments without oxygen (anoxic). The fourth group is very sensitive to oxygen. These strains, called **strict anaerobes**, cannot grow in the presence of any oxygen and must be cultured under special conditions to exclude any air from the growth medium. A major difference between bacteria that tolerate oxygen and those that are killed by it is the presence of enzymes in the tolerant stains that protect against toxic oxygen molecules such as peroxide, superoxide, singlet oxygen, and oxygen radicals.

Most of the strains used in the classroom either require oxygen for growth or grow better with oxygen. **These bacteria will grow best on agar plates, where air readily diffuses into the bacterial colony, or in liquid cultures that are shaken**. Since diffusion of oxygen into liquid depends on the surface area, it is important to have a large surface:volume ratio. This means that cultures will grow best in flasks in which the volume of liquid is small relative to the size of the vessel. For best aeration, no more than 10%-20%of the total volume of the vessel should be liquid. A growing bacterial culture quickly depletes the medium of all oxygen, so that a culture will become anoxic even if it is exposed to air. Rapid shaking of a culture with a large surface:volume ratio is required to provide sufficient oxygen for respiration. Without rapid

shaking and a large surface area, the bacteria will grow aerobically until they reach moderate populations but will then switch to fermentation as oxygen becomes limiting.

The final factor that affects growth is the nutritional medium. Bacteria grow best when optimal amounts of nutrients are provided; however, the nutritional needs of bacteria vary tremendously. Some strains require a nutritionally rich medium full of amino acids, peptides, vitamins and sugar. This rich broth would kill other bacteria. Nutrient broth is a moderately rich medium that allows good growth of most of the bacteria used in the classroom. It lack sugars, which increase the growth rate but also increase the death rate because the metabolism of sugars produces acids that kill the cells. Minimal medium, which provides only the essentials that will allow many bacteria to make their own amino acids and vitamins, is often used in the lab; however, bacteria growing in minimal medium have a long lag phase and they grow slowly.

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