Learning Outcomes:

At the end of this lecture, the students should be able to:

1. Describe the process of synthesis bacterial cell wall
2. Define L forms
3. Identify the chemical and environmental factors affecting bacterial growth
4. Explain how microbes are classified on the basis of O₂ needs
5. Explain the bacterial growth curve

Cell wall growth

Cell wall synthesis is necessary for cell division; however, the incorporation of new cell wall material varies with the shape of the bacterium. Rod-shaped bacteria (eg, *E. coli, Bacillus subtilis*) have two modes of cell wall synthesis; new peptidoglycan is inserted along a helical path leading to elongation of the cell, and is inserted in a closing ring around the future division site, leading to the formation of the division septum. Coccoid cells such as *S. aureus* do not seem to have an elongation mode of cell wall synthesis. Instead, new peptidoglycan is inserted only at the division site. A third form of cell wall growth is exemplified by *S. pneumoniae*, which are not true cocci, as their shape is not totally round, but instead have the shape of a rugby ball. *S. pneumoniae* synthesize cell wall not only at the septum but also at the so-called equatorial rings (Figure 1).
Protoplasts, Spheroplasts, & L Forms

Removal of the bacterial wall may be accomplished by hydrolysis with lysozyme or by blocking peptidoglycan synthesis with an antibiotic such as penicillin. In osmotically protective media, such treatments liberate protoplasts from gram-positive cells and spheroplasts (which retain outer membrane and entrapped peptidoglycan) from gram-negative cells.

If such cells are able to grow and divide, they are called L forms. L forms are difficult to cultivate and usually require a medium that is solidified with agar as well as having the right osmotic strength. L forms are produced more readily with penicillin than with lysozyme, suggesting the need for residual peptidoglycan.

Some L forms can revert to the normal bacillary form upon removal of the inducing stimulus. Thus, they are able to resume normal cell wall synthesis. Others are stable and never revert. The factor that determines their capacity to revert may again be the presence of residual peptidoglycan, which normally acts as a primer in its own biosynthesis.

Some bacterial species produce L forms spontaneously. Lead to chronic infection and they resist antibiotic treatment and give us a great problem in healing the focus of infection.
Figure 1: Incorporation of new cell wall in differently shaped bacteria. Rod-shaped bacteria such as *Bacillus subtilis* or *Escherichia coli* have two modes of cell wall synthesis: new peptidoglycan is inserted along a helical path (A), leading to elongation of the lateral wall, and is inserted in a closing ring around the future division site, leading to the formation of the division septum (B). *Streptococcus pneumoniae* cells have the shape of a rugby ball and elongate by inserting new cell wall material at the so-called equatorial rings (A), which correspond to an outgrowth of the cell wall that encircles the cell. An initial ring is duplicated, and the two resultant rings are progressively separated, marking the future division sites of the daughter cells. The division septum is then synthesized in the middle of the cell (B). Round cells such as *Staphylococcus aureus* do not seem to have an elongation mode of cell wall synthesis. Instead, new peptidoglycan is inserted only at the division septum (B).

**Nutritional requirement for microorganisms**

The essential elements for the bacterial growth in the body of the host or on culture media are:

**1-Carbon source:** Chemolithotrophs, organisms that use an inorganic substrate such as hydrogen or thiosulfate as a reductant and carbon dioxide as a carbon source. Heterotrophs require organic carbon for growth, and the organic carbon must be in a form that can be assimilated.
2- Nitrogen source: Most microorganisms can use NH$_3$ as a sole nitrogen source, and many organisms possess the ability to produce NH$_3$ from amines (R—NH$_2$) or from amino acids. Many microorganisms possess the ability to assimilate nitrate (NO$_3^-$) and nitrite (NO$_2^-$) reductively by conversion of these ions into NH$_3$. A few bacteria use N$_2$ in nitrogen fixation, a process by which nitrogen (N$_2$) in the atmosphere is converted into ammonia (NH$_3$).

3- Sulfur Source: Sulfur in its elemental form cannot be used by plants or animals. However, some autotrophic bacteria can oxidize it to sulfate (SO$_4^{2-}$). Most microorganisms can use sulfate as a sulfur source, reducing the sulfate to the level of hydrogen sulfide (H$_2$S). Some microorganisms can assimilate H$_2$S directly from the growth medium, but this compound can be toxic to many organisms.

4- Phosphorus Source: Phosphate (PO$_4^{3-}$) is required as a component of ATP, nucleic acids, and such coenzymes as NAD and NADP. In addition, many metabolites, lipids (phospholipids, lipid A), cell wall components (teichoic acid), some capsular polysaccharides, and some proteins are phosphorylated. Phosphate is always assimilated as free inorganic phosphate (P$_i$).

5- Mineral Sources: Numerous minerals are required for enzyme function. Mg$^{2+}$ and K$^+$ are both essential for the function and integrity of ribosomes. Ca$^{2+}$ is required as a constituent of gram-positive cell walls, though it is dispensable for gram-negative bacteria. Many marine organisms require Na$^+$ for growth.

6- Growth Factors: are organic compounds obtained from the environment, where cells must have them in order to grow, but unable to synthesize them, like vitamins.
Environmental factors affecting growth

1. **Nutrients**

2. **pH**: each organism need a certain PH for its growth.

   - Neutrophiles 6-8,
   - Acidophiles 3
   - Alkalophiles 10.5

   Majority of microorganisms grow at a pH between 6-8

3. **Temperature**: Different microbial species vary widely in their optimal temperature ranges for growth: Psychrophilic (15-20\(^{0}\) C), Mesophilic (30-37\(^{0}\) C), Thermophilic (50-60\(^{0}\) C).

   Some organisms are hyperthermophilic and can grow at well above the temperature of boiling water, which exists under high pressure in the depths of the ocean. Most organisms are mesophilic; 30\(^{0}\)C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.

4. **Oxygen (O2)**: most bacteria require O2 necessary (strict aerobes). Others can live with or without O2: Facultative bacteria as *E.coil*. Other requires a small amount of O2: (microaerophilic). The rest cannot grow in presence of O2: anaerobic bacteria e.g., *Clostridium*.

5. **Osmotic Pressure**: cytoplasmic membrane and cell wall protect bacteria cell from varying contents of salts in the medium. For most species the maximum conc. of salts is (5-12%), some other species can grow in high conc. (osmophilic bacteria) e.g: *Staph. aureus*. Organisms requiring high salt concentrations are called halophilic; those requiring high osmotic pressures are called osmophilic.
**Bacterial Growth**

Bacteria reproduce by binary fission, a process by which one parent cell divides to form two progeny cells.

When a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at frequent intervals, the typical phases of a standard growth curve can be demonstrated.

The number of bacteria in a medium could be:

1- A total cell count curve is based on the number of cells present that are alive or dead.

2- A viable cell count curve measures only living (viable) cells (capable of growing and producing a colony on a suitable growth medium).

The typical phases of a standard growth curve are (Figure 2):

1- **Lag phase:** during vigorous metabolic activity occurs but cells do not divide. This can last for a few minutes up to many hours.

2- **Log (logarithmic) or exponential phase:** is when rapid cell division occurs. There is a linear relationship between time and log of number of cells. β-Lactam drugs, such as penicillin, act during this phase because the drugs are effective when cells are making peptidoglycan (i.e., when they are dividing).

3- **Stationary phase:** occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state.

4- **Death or decline phase:** decline in the number of viable bacteria.
Summary

1. L-forms are bacterial variants that lack a cell wall

2. Nutrients: Macronutrients/micronutrients

3. Generation time: Time required for cell to divide/for population to double

4. The growth cycle of a culture of bacteria is divided into four phases: lag phase, exponential phase, stationary phase, decline phase

Main References:
