Growth and Multiplication of Bacteria
There are four phases of bacterial growth [and death]: the lag phase is in black; the log phase is in blue; the stationary phase is in red; the death or decline phase is in green.
The use of Durham tubes: tiny tubes placed in solutions of peptone water with varying kinds of carbohydrates. They are placed upside down in the liquid medium. In the illustration **BLUE** is the color of the medium that it starts with. **GREEN** indicates opacity or bacterial growth in the medium. **RED** indicates that acid has been produced. The **blue squiggly circles** represent gas bubbles formed by bacteria that are fermenters. Each of these changes are used to identify different bacteria.
• An anaerobic jar: This jar is sealed after samples are placed in it with an indicator (methylene blue -- clear is reduced) or even Pseudomonas aeruginosa (if it grows, the jar is NOT anaerobic)).
• The valve (circle with "X" in it) is opened to permit a vacuum to remove air from it. Once that is complete, the valve is turned, again, and hydrogen is pumped into it, rendering the atmosphere in the jar anaerobic. The valve is turned to seal it from all gas sources, then the jar is incubated.
• An alternative to anaerobic jars: the infamous candle jar. This is handled just like the anaerobic jar EXCEPT that after samples are placed in the jar, a lit candle is, too. The lid is replaced tightly and the candle burns out, using all the oxygen in the jar, permitting enough carbon dioxide to be present to make 10-15% CO₂ in the jar. The jar is then incubated.

• Another alternative to the anaerobic and candle jars is to place your sample in a zip-loc bag with some baking soda and vinegar in separate parts of a Petri dish. Seal the bag, pressing out the air as you seal the bag. Once sealed, tilt the Petri dish so that the baking soda and vinegar may mix, releasing carbon dioxide in the bag, creating an anaerobic environment for your microbes to grow in.
• Isolation of pure cultures: pure culture = only one kind of bacteria present.
• Generally from a mixed culture (consists of more than one kind of bacteria present in culture).
• Classical method is by obtaining these colonies by plating on solid medium.
Flame Alternative

2. Plug cord in to outlet.
3. Turn switch to the “on” position (it will light up).
4. Allow to heat up for at least 15 minutes – at that time, the interior of the chamber is 816° C!!!
5. Obtain an inoculating loop with an insulated handle and carefully insert it inside the ceramic chamber – do not scrape the sides of the ceramic chamber with the loop – it can split.
6. Hold the loop in the back of the chamber for a minimum of 10 seconds.
7. The loop need not glow red as it does in a Bunsen burner to be sterilized.
9. Use loop to obtain bacterial sample, streak or smear media or slide, then repeat steps 4-7 and set aside.
10. Repeat steps 4-7 every time you use the loop.
11. Turn off the Bacti-Cinerator IV at the end of the lab period or if everyone is done using it.
A simple manner in which bacteria can be plated out to isolate them as discrete colonies from confluently grown together plaques. A flame (or Bacti-Cinerator) sterilized loop is cooled to room temperature. A sample of bacteria is removed from another plate and streaked across a small portion of the new agar plate (A). The flame is then re-sterilized, cooled, then streaked across A into B as illustrated. Repeat the process from B to C. After the loop has been re-sterilized following inoculation of C, run a single streak from C into D and incubate after you've sterilized the loop, again.

A variation of this is to streak a swab saturated with a patient's sample across area A, then repeat as above with a flame sterilized loop.
• Regardless of the method, from the resulting culture, it is then possible to pick out single colonies of dissimilar bacteria and grow on other media for identification purposes.
Conditions for bacterial growth:

• Bacteria require water, inorganic salts, carbon and nitrogen sources, growth factors and a source of energy.

• Additionally, their growth depends on the redox potential, pH and temperature.
• Water: Dessication kills bacteria, although, in special cases they may survive for periods in a suspended animation state. Common method of preserving lab specimens is to lyophilize them and store them in vacuo.
• Inorganic salts: These are required for osmotic regulation and to provide trace elements necessary for certain enzyme systems. All bacteria require phosphate. Sulfate is essential if there is no other source of S. Na, K, Mg, Ca, Fe, Mn, Zn, Cu, Co, Mo are also required.
• Carbon, nitrogen, growth factors and energy: two main groups;
• Autotrophs and
• Heterotrophs.
• **Autotrophs** are capable of living in a strictly inorganic environment. They have no direct medical importance.

• These bacteria obtain their C from CO$_2$ and N from NH$_3$, NO$_2^-$ and NO$_3^-$. A few are photosynthetic and obtain their energy from light by means of chlorophyll.

• Most are chemosynthetic and obtain their energy by oxidizing substances such as NH$_3$, NO$_2^-$, Fe$^{2+}$, S$_2^-$, S and H.

• They manufacture all the complicated proteins, carbohydrates, lipids, nucleic acids and enzymes needed for growth and metabolism.
• **Heterotrophs** require preformed organic matter for energy and synthesis.

• **All bacteria of medical importance come into this category.**

• In general, heterotrophs obtain their C, N and energy from organic compounds such as carbohydrates and amino acids.

• Many pathogenic species do not synthesize certain key substances such as vitamins, purines and pyrimidines.

• These organisms grow when they receive ready-made growth factors. E.g.,
  
  – most complicated: Streptococci require 17 amino acids, 9 B vitamins, adenine and guanine, cytosine, thymine and uracil and a carbohydrate for energy;
  
  – least complicated: E. coli obtains N from NH₃ but requires a complex source of C such as glucose.
- **Gaseous requirements:**
- **CO₂**: all bacteria require CO₂ for metabolism. Growth of nearly all micro-organisms, particularly N. gonorrhoeae, N. meningitides, Streptococci is improved by the presence of additional CO₂.
- **O₂**: Bacteria may be classified into four groups based upon their usage of oxygen:

<table>
<thead>
<tr>
<th>Obligate Aerobes</th>
<th>[Strict] Anaerobes</th>
<th>Facultative Anaerobes</th>
<th>Microaerophiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grow only with O₂</td>
<td>Grow only without O₂; killed by O₂</td>
<td>Will grow under either aerobic or anaerobic conditions</td>
<td>Grow best with lower O₂ concentrations (ca 10%)</td>
</tr>
<tr>
<td>M. tuberculosis; P. aeruginosa</td>
<td>Clostridium; some Strep/Staph; [Spirochaetes]</td>
<td>Nearly all organisms of medical importance NOT in the first 2 groups</td>
<td>Some Streptococci; Mycoplasma</td>
</tr>
</tbody>
</table>
Growth of 4 types of microorganisms: **Strict aerobes** which grow in agar shakes (heat the agar above 50° C to melt it. At 50°C, add bacteria, mix it carefully, put a plug in the top to keep dirt, etc out, then let it incubate) grow at the top of the shakes. **Strict anaerobes** grow at the bottom of the shake, where the environment is the most anaerobic. **Facultative anaerobes** grow throughout the medium as they can grow in both aerobic and anaerobic environments. **Microaerophiles** prefer to grow just below the surface of the medium where the oxygen concentration is only about 10% or so as opposed to the 21% in atmospheric air.
• Shake cultures are tubes of freshly melted agar, cooled to 50° C and inoculated.

• Anaerobes will grow under AEROBIC conditions in liquid media if they contain sufficient reducing substances: e.g., sodium thioglycollate, ascorbate, couple of dried peas or an iron nail. Cooked meat medium is the best anaerobic medium, however.
• Hyperbaric oxygen (O₂ at pressures greater than atmospheric pressure) has been used in the treatment of anaerobic infections, notably gas gangrene.

• Nitrogen: gaseous nitrogen is not required by bacteria of medical importance.

• Nitrogen fixating bacteria are important in agriculture.
• pH: most bacteria of medical importance grow best between pH 7.2 and 7.6.
• Growth is usually poor below 6 or above 7.8.
• Growth ceases below 5 or above 9.
• Exceptions are V. cholerae (8-9) and L. acidophilus (4).
Temperature: Bacteria pathogenic for man usually grow best at body temperature, 37°C. Occasionally, this temperature is higher (C. jejuni – 43°C, M. avium – 40°C) or lower (Y. pestis – 30°C, M. ulcerans – 32°C). Although many species will multiply over a range of 20-43°C, some species like N. gonorrhoeae will grow only in a narrow range around 37°C.

a. Bacteria encountered in medical bacteriology are mesophilic in their temperature requirements.

a. Some psychrophilic (cold loving) species found in brine and soil will multiply at 0°C or lower.

a. Some thermophilic species found in hot springs and manure piles will multiply at temperatures as high as 55-80°C. In hydrothermal vents where water is under high pressure, certain species grow at temperatures above 100°C.
• Metabolism: the main metabolic pathways are the same as those utilized by other forms of life. Bacteria may utilize many different substrates. The end-products they produce show amazing diversity. The rate of metabolism is unusually rapid. This is due to the large surface area:volume, which facilitates the exchange of nutrients and wastes.
Carbohydrates are the major source of energy for medically important bacteria. Two types of bacteria:

1) Homofermentative: oxidize glucose by glycolysis to produce lactate as the main end product, e.g., Streptococcus and Lactobacillus.

1) Heterofermentative: produce varying amounts of other end-products.

Many bacteria carry out the intermediate reactions of the TCA (as previously discussed in BIOL 190, 223)) cycle and various reactions with pyruvate and 2 carbon fragments. These reactions serve synthetic as well as catabolic processes and act as a bridge between carbohydrate metabolism and the metabolism of proteins and lipids. Carbohydrate media may be used for biochemical tests. Sugar media: fermentative patterns are important in bacterial identification.

REMEMBER:

Fermentation: $\text{CHO} \rightarrow \text{EtOH} + \text{CO}_2$

Oxidation: $\text{CHO} \rightarrow \text{CO}_2 + \text{H}_2\text{O}$
Aerobic Growth

In aerobic respiration, the H\(^+\) derived from oxidative processes are transferred to molecular oxygen by means of the cytochromes. Glucose is completely catabolized to carbon dioxide and water and maximum energy is liberated.

In fermentation, the H\(_2\) is transferred to other hydrogen acceptors or is liberated as H\(_2\)↑.

Depending on their use of these 2 types of oxidation, bacteria fall into 3 broad groups:

1) obligate aerobes – possess cytochromes and carry out aerobic respiration
2) Streptococcus, Lactobacillus, with rare exceptions, obligate anaerobes do not have cytochromes and carry out only fermentations
3) The great majority of organisms have cytochrome systems which they use when oxygen is available, but adapt to fermentative processes when oxygen is limited or absent.
Media Composition

• Synthetic media: chemically defined media comes from pure substances (AA’s, growth factors, salts, etc). Media used in the lab usually contains a mixture of naturally occurring biological substances and their partial “breakdown”, i.e., hydrolytic products. Peptone, meat extract and salt provide the basis for most media. Peptone is a complex mixture of water soluble products obtained by enzymatic digestion of meat (pepsin). Whole blood, heated blood, serum, yeast extract, glucose and glycerol may be added to increase nutritive value.

• Different components effect the way bacteria grow or don’t grow. Selective media encourages or inhibits the growth of some specific bacteria. Differential media makes it possible to differentiate between organisms. Media making has already been covered in the laboratory.
<table>
<thead>
<tr>
<th>Media</th>
<th>Composition</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>Consists of peptone, meat extract and NaCl</td>
<td>General usage</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>Nutrient broth plus agar</td>
<td>General usage</td>
</tr>
<tr>
<td>Blood agar</td>
<td>Nutrient agar with 5-10% citrated or oxalated or defibrinated blood – may be horse or sheep – sheep more common</td>
<td>Most bacteria of medical importance will grow on this medium. Presence of intact RBC allows for detection of hemolytic properties of organisms</td>
</tr>
<tr>
<td>Chocolate agar</td>
<td>Blood agar that has been heated until it is a chocolate color. This increases the nutritive value.</td>
<td>Delicate organisms such as N. gonorrhoeae.</td>
</tr>
<tr>
<td>Cooked meat medium</td>
<td>Minced meat suspended in broth. Has excellent nutritive properties and supports the growth of a large number of organisms.</td>
<td>Great for strict anaerobes.</td>
</tr>
<tr>
<td>Media</td>
<td>Composition</td>
<td>Usage</td>
</tr>
<tr>
<td>------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cystine-Lactose-Electrolyte-Deficient medium (CLED)</td>
<td>Cystine allows for growth of cys-cys dependent organisms; lactose and bromothymol blue (BTB) for differential properties; lyte deficient to prevent swarming of Proteus.</td>
<td>Lactose fermenters (E. coli) produce yellow colonies; non lactose fermenters (Proteus) produce blue colonies.</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>Contains peptone as the main source of nutriment; bile salts which have a weak suppressive effect on non-intestinal bacteria; lactose and neutral red which confer differential properties on the medium.</td>
<td>Most important differential medium for general purposes such as examination of urine and wound swabs. Lactose fermenters (E. coli) produce pink colonies; Salmonella and Shigella (intestinal pathogens) produce colorless colonies. S. faecalis and S. aureus (fermenters) and Proteus and P. aeruginosa (non-fermenters) grow as well. S. pyogenes is inhibited.</td>
</tr>
<tr>
<td>Media</td>
<td>Composition</td>
<td>Usage</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bacterial transport medium</td>
<td>Contains salts, sodium thioglycollate to provide anaerobic conditions,</td>
<td>Used to encourage survival of delicate organisms such as N. gonorrhoeae and T. vaginalis when there is delay in transporting the specimens to the lab for analysis.</td>
</tr>
<tr>
<td></td>
<td>methylene blue to check that these conditions are maintained (colorless and sufficient agar (ca. 0.3%) to render the medium semi-solid. The specimen is taken with charcoal impregnated/coated swabs which are free of inhibitory substances found in cotton wool. Swabs are inserted in the medium, snapped off and the bottle cap screwed on tightly.</td>
<td></td>
</tr>
</tbody>
</table>
