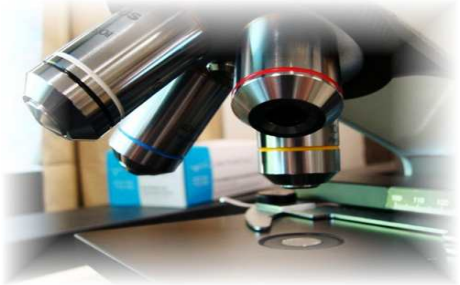

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• The SPO Virtual Classrooms offer many educational resources, including practice test questions, review questions, lecture PowerPoints, video tutorials, sample assignments and course syllabi. New materials are continually being developed, so check back frequently, or follow us on Facebook (Science Prof Online) or Twitter (ScienceProfSPO) for updates.

• Many SPO PowerPoints are available in a variety of formats, such as fully editable PowerPoint files (.ppt), as well as uneditable versions in smaller file sizes, such as PowerPoint Shows (.pps) and Portable Document Format (.pdf), for ease of printing. The font "Jokerman" is used frequently in titles. It has a microbiology feel to it. If you do not have this font, some titles may appear odd, oversized and off-center. Find free downloads of Jokerman by Googling "download jokerman font microsoft".

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• Several helpful links to fun and interactive learning tools are included throughout the PPT and on the Smart Links slide, near the end of each presentation. You must be in *slide show mode* to utilize hyperlinks and animations.

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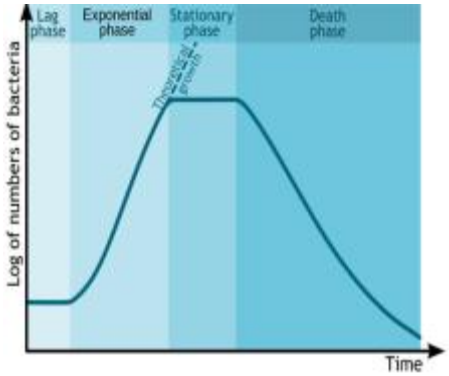
Image: Compound microscope objectives, T. Port



Microbial Growth & Metabolism

Image: : MacConkey's media, clockwise from top left – *E. coli, Enterobacter, Salmonella;* & Mannitol Salt Agar (MSA), T. Port; Bacterial growth phases, M. Komorniczak

From the Virtual Microbiology Classroom on ScienceProfOnline.com

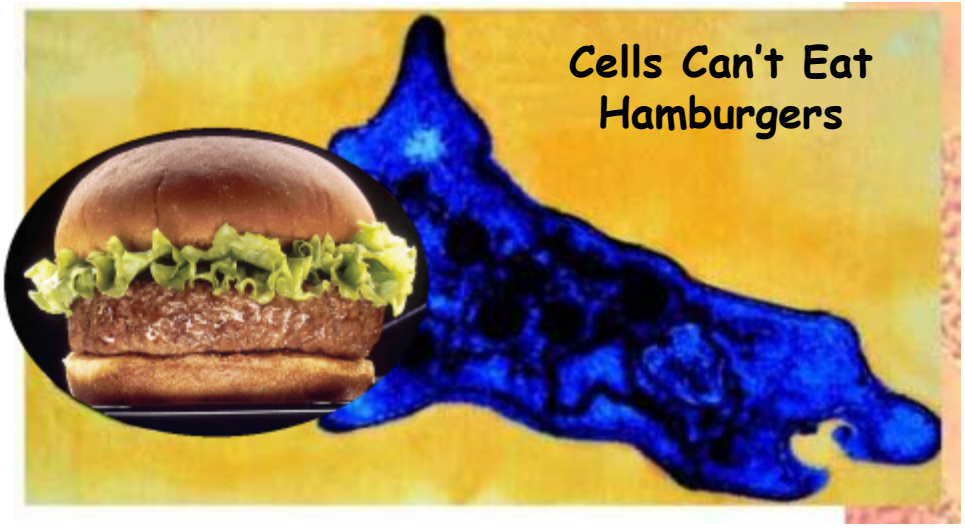
Metabolism

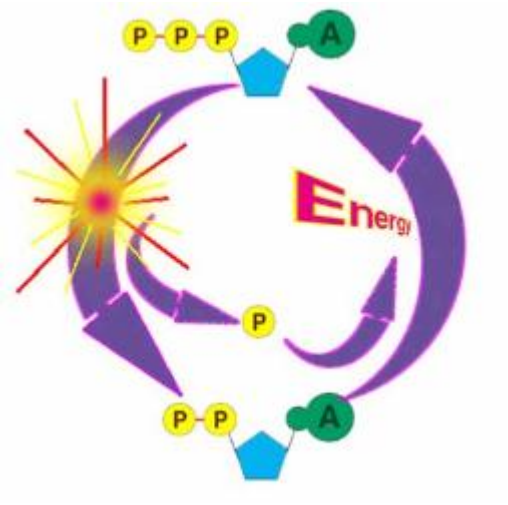
The Transformation of Energy

- Cells either get their energy either by **photosynthesis** or by **eating stuff**.

- But a cell can't just use sunlight or nutrients to run cellular reactions.

Q: What type of fuel is needed to run a cell?





The mother of all rechargeable batteries.

Images: Hamburger, Wiki; ATP-ADP Cycle, CUNY

Building and Breaking Down Molecules

Anabolic Reaction

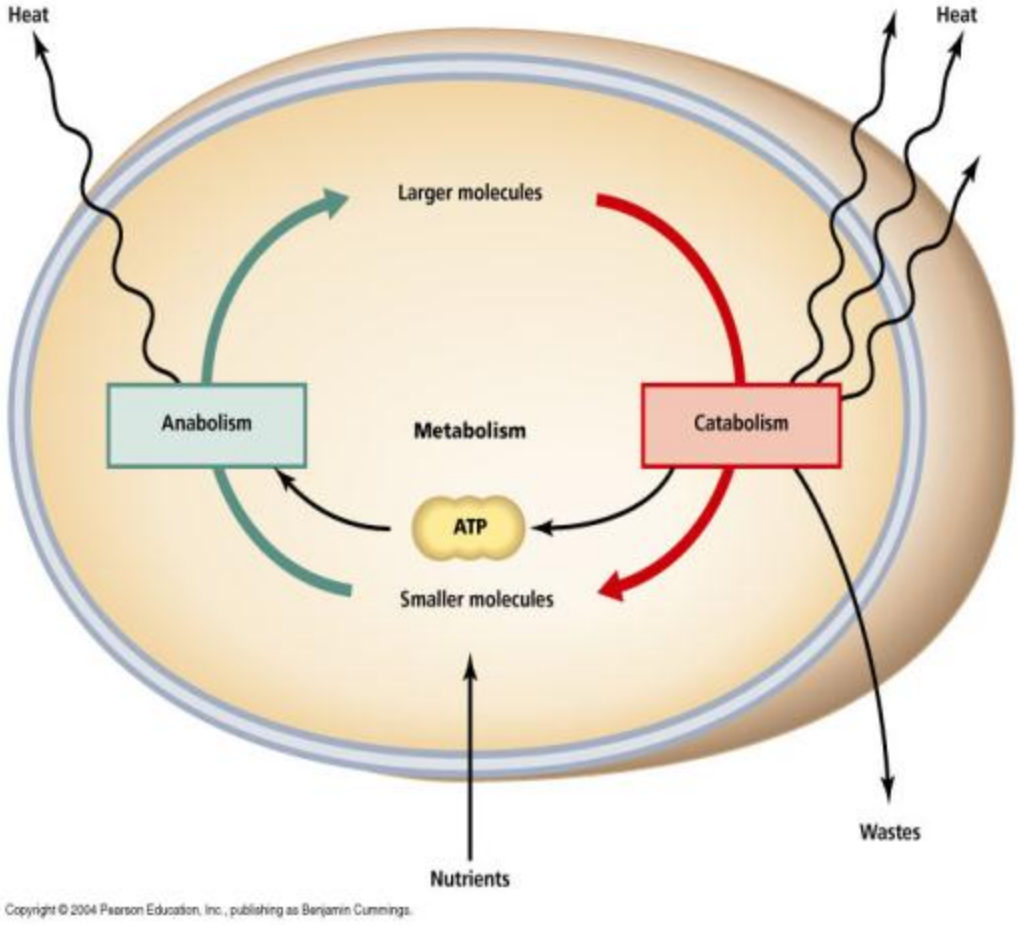
(anabolism)

The phase of metabolism in which simple substances are **synthesized** into the complex materials of living tissue.

Catabolic Reaction

(catabolism)

The metabolic **break down** of complex molecules into simpler ones, often resulting in release of energy.



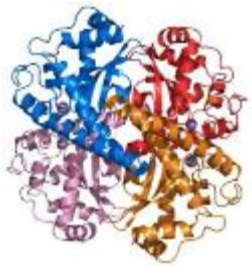
Cellular Respiration *is* Catabolism

- Organisms **catabolize** (break down) **carbohydrates** as the primary energy source for anabolic reactions.
- The monosaccharide **glucose** is used most commonly.
- Glucose catabolized by:
  - **Aerobic cellular respiration** → Requires oxygen. Results in complete breakdown of glucose to carbon dioxide, water and a lot of

  - **Anaerobic cellular respiration** → Does not require oxygen, but does require and oxygen "stand in". Only partially breaks down glucose, so makes less

Q: *What is required for respiration to be **aerobic**?*

Microbes & Oxygen



Using oxygen (1/2 O_2) in metabolism creates toxic waste.

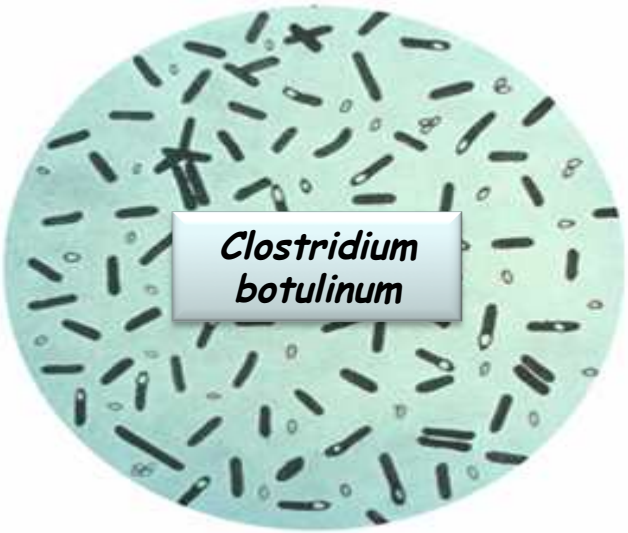
Microbes that are able to use aerobic respiration produce enzymes to detoxify oxygen:

Catalase: H_2O_2 ---→ H_20 and 0_2
Superoxide dismutase (SOD): oxygen radical ---→ H_20 and O_2

Microbes that don't make these enzymes cannot exist in the presence of oxygen.

Image: Catalase enzyme structure, Vossman; Superoxide dismutase enzyme structure, Fvasconcellos

Bacterial Genus: *Clostridium*



GRAM-POSITIVE
Obligate anaerobe, bacillus-shaped

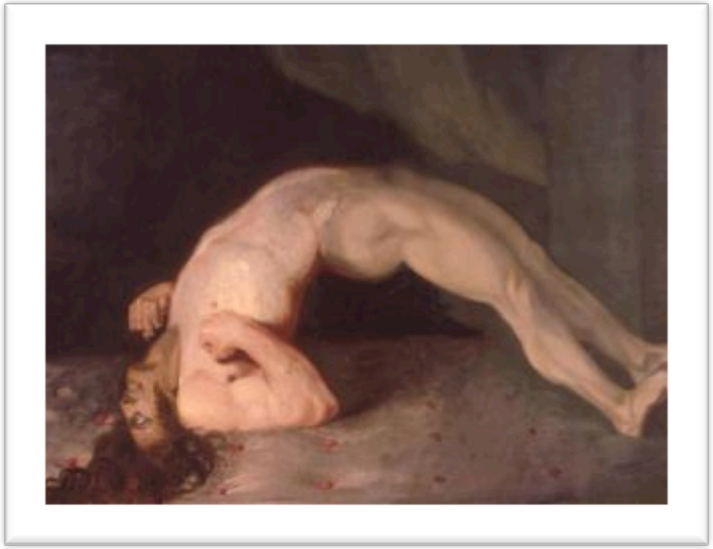
All species form endospores.

All have a strictly fermentative mode of metabolism (Don't' use oxygen).

Vegetative cells are killed by exposure to O^2, but their endospores are able to survive long periods of exposure to air.

Known to produce a variety of toxins, some of which are fatal.

Clostridium tetani = agent of tetanus
C. botulinum = agent of botulism
C. perfringens = one of the agents of gas gangrene
C. difficile = part of natural intestinal flora, but resistant strains can overpopulate and cause pseudomembranous colitis.



Images:Clostridium botulinum: stained with Gentian violet. CDC Public Health Image Library. (PHIL #2107), 1979; Charles Bell 1809 painting.

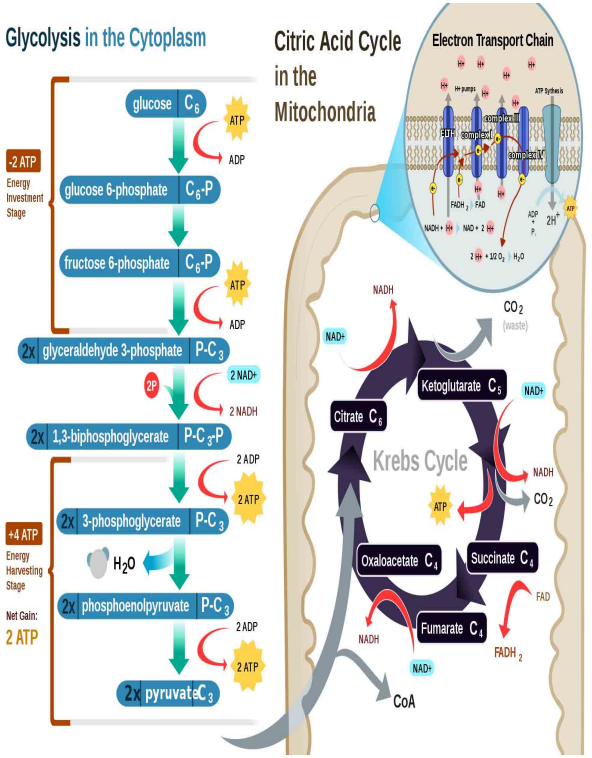
Cellular respiration →

The steps that a cell must go through to turn other forms of energy into ATP.

The 4 subpathways of cellular respiration are …

1. glycolysis
2. synthesis of acetyl-CoA
3. Krebs cycle
4. electron transport chain ETC

…which result in complete breakdown of glucose to carbon dioxide, water and ATP



When food is broken down, electrons from the food are transferred to other molecules that move through cellular respiration pathways. This leaves "left over" electrons that need to be disposed of at the end of the ETC .

Q: *What is required for respiration to be* ***aerobic?***

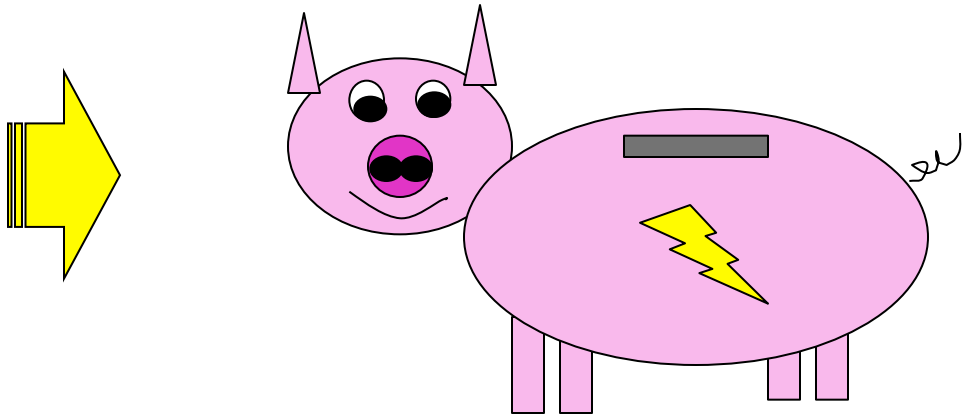
Images: Cellular Respiration, Regis Frey

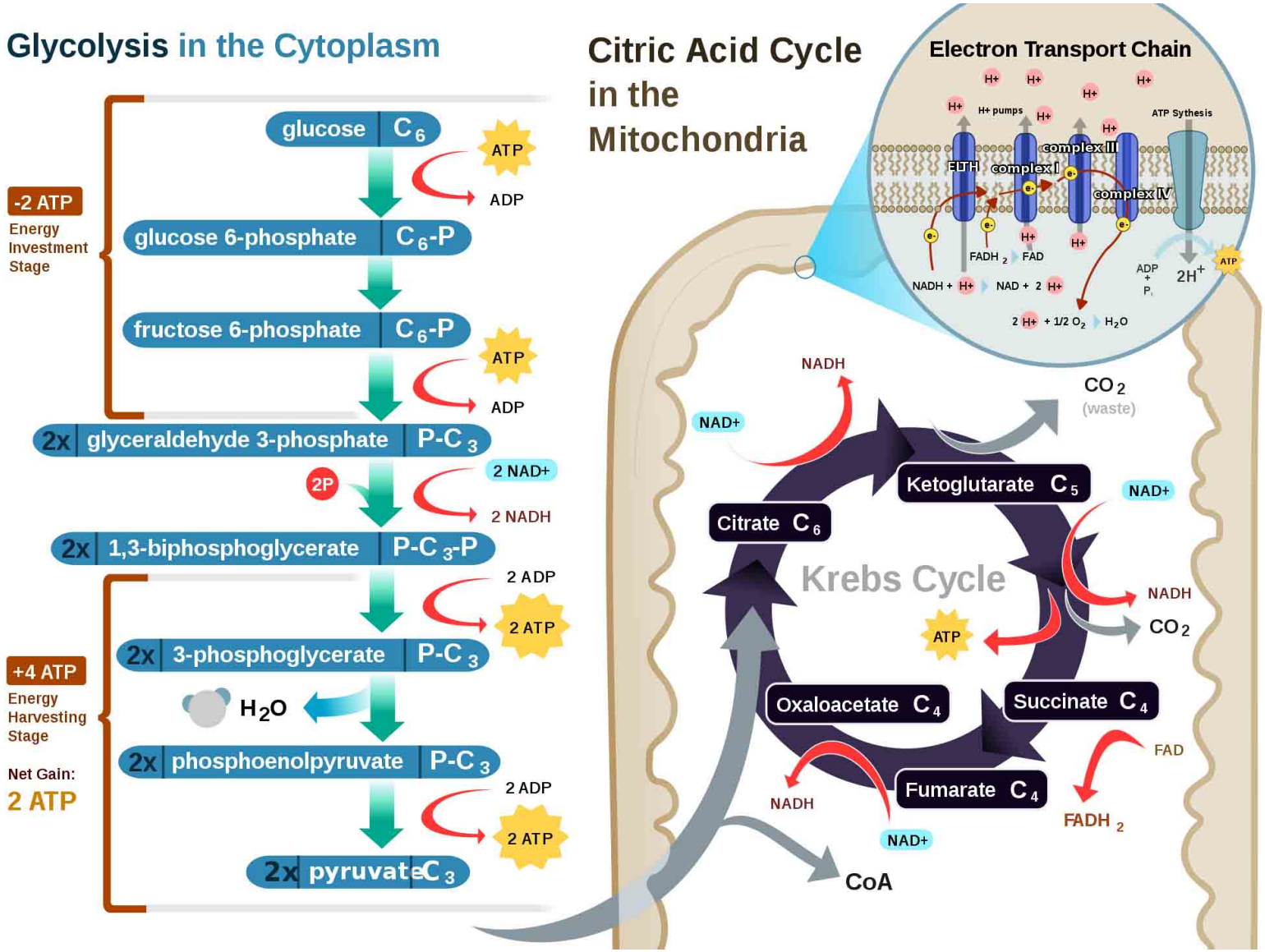
From the Virtual Microbiology Classroom on ScienceProfOnline.com

Aerobic Cellular Respiration

Subpathway	Molecule In	Molecule Out	Energy Obtained
1. glycolysis			
2. synth acetyl-CoA			
3. Krebs cycle			
4. ETC			

Let's put the energy extracted from glucose into our energy piggy bank.



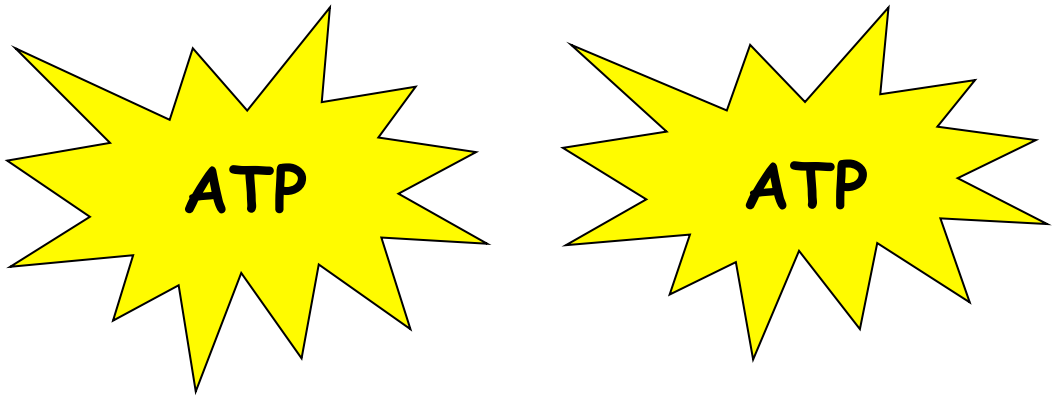


Images: Cellular Respiration, Regis Frey

From the Virtual Microbiology Classroom on ScienceProfOnline.com

Fermentation

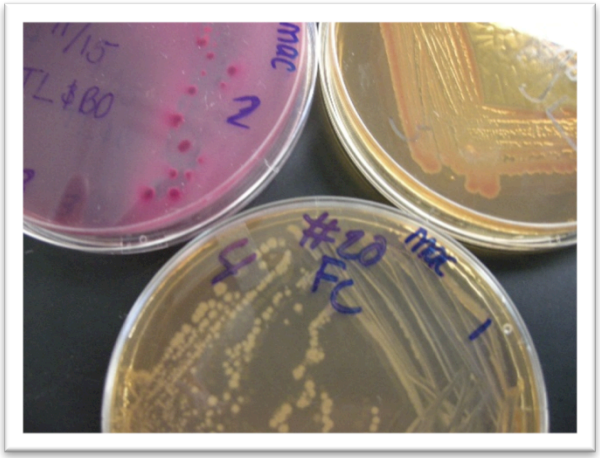
- When there is nothing that can "catch" the electrons at the end of the ETC, cellular respiration cannot happen.
- Fermentation is an alternative system that allows glycolysis to continue without the other steps of cellular respiration.
- Not as energetically efficient as respiration.
- Produces only 2 ATP.



Metabolism & Identification of Microbes

Some of the specialized media that we have worked with in lab is both **selective** and **differential**.

The **differential** properties give us information about bacteria based on its metabolism.



Qs: *What is the medium in top picture?*

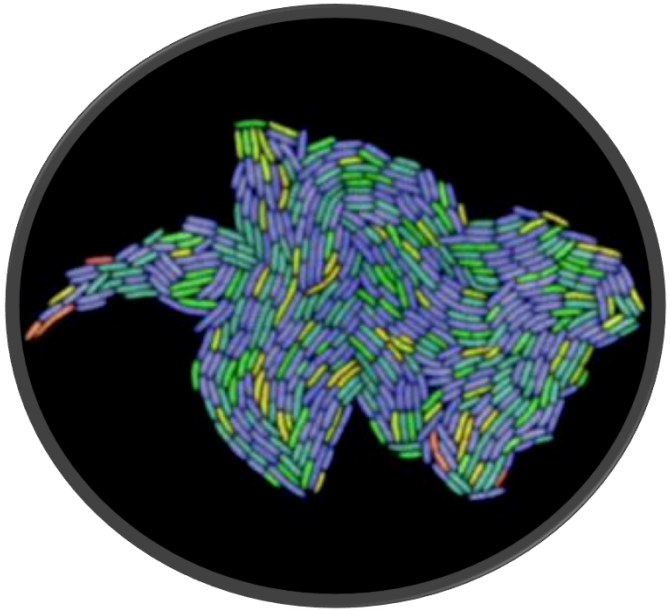
- *Is selective …Why? What does it grow?*
- *Is differential …Why?*
- *What does the differential property reveal about the bacteria growing there?*

Qs: *What is the medium in bottom picture?*

- *Is selective …Why? What does it grow?*
- *Is differential …Why?*
- *What does the differential property reveal about the bacteria growing there.*

Images: MacConkey's Agar (MAC) & Mannitol Salt Agar (MSA), T. Port

Microbial Growth



- Refers to increase in the **number** of microbes (reproduction) rather than an increase in **size** of the microbe.

- Result of microbial growth is the **colony** = aggregation of cells arising from single parent cell.

- The time required for growth and reproduction is known as the doubling or **generation time**.

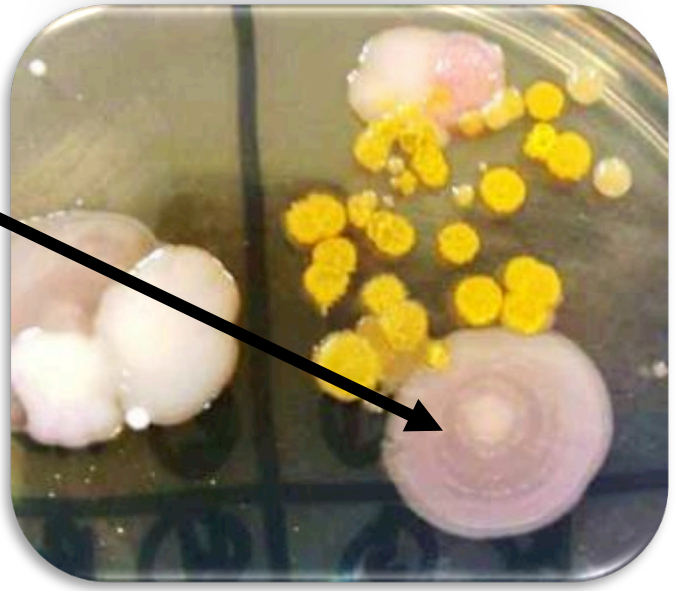


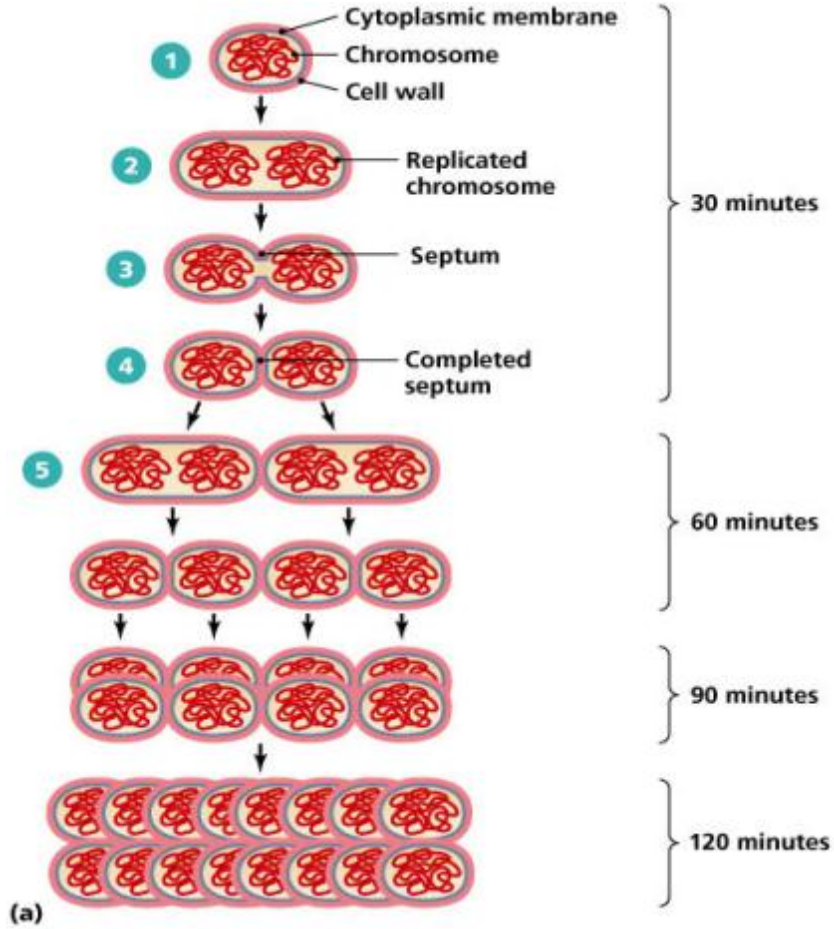
Image: Glowing Colony E. coli from "Aging and Death in E. coli" (2005) PLoS Biol 3(2); Microbes on MacConkeys, T. Port

From the Virtual Microbiology Classroom on ScienceProfOnline.com

Exponential Growth in Cell Count From Binary Fission

Generation Number	Cell Count
0	1
1	2
2	4
3	8
4	16
5	32
10	1,024
20	1,048,576

Let's watch a time lapse movie of *E. coli* population growth.



From the Virtual Microbiology Classroom on ScienceProfOnline.com

Bacterial Population Growth Curve

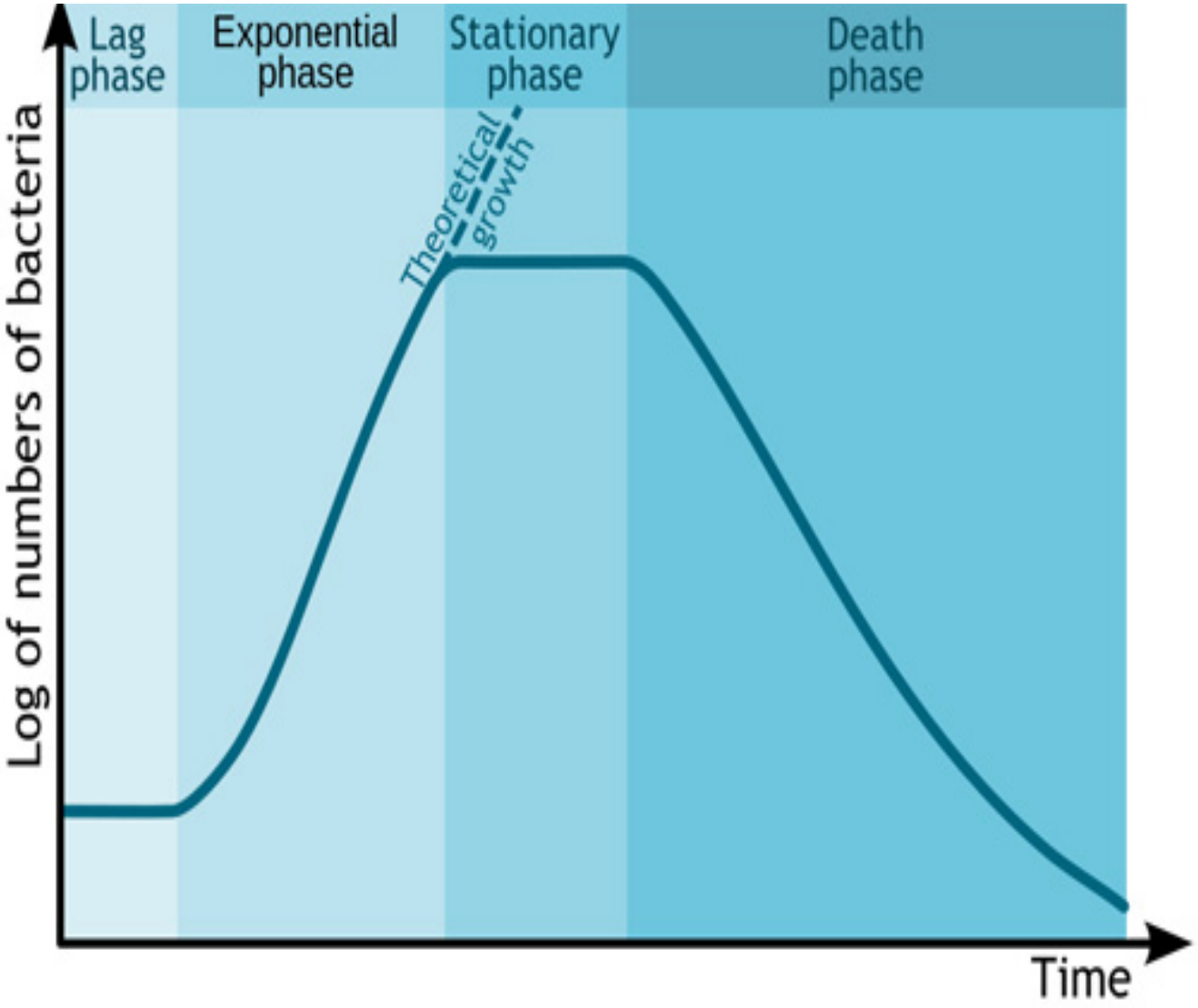


Image: Bacterial growth phases, Michal Komorniczak

Generation Time Under Optimal Conditions

(at 37°C)

Organism	Generation Time	
Bacillus cereus	28 min	
Escherichia coli	12.5 min	
Staphylococcus aureus (causes many types of infections)	27-30 min	
Mycobacterium tuberculosis (agent of Tuberculosis)	18 – 24 hrs	
Treponema pallidum (agent of Syphilis)	30 hrs	

Images: *B. cereus, E. coli* & *S. aureus* by T. Port;
TB culture, Dr. George Kubica PHIL #4428,
Treponema pallidum, Dr. Edwin P. Ewing, Jr., PHIL

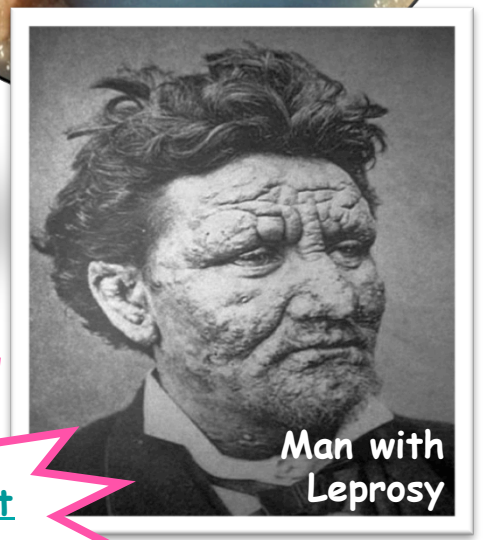
Bacterial Genus: *Mycobacterium*

GRAM-variable, obligate aerobe, bacillus-shaped

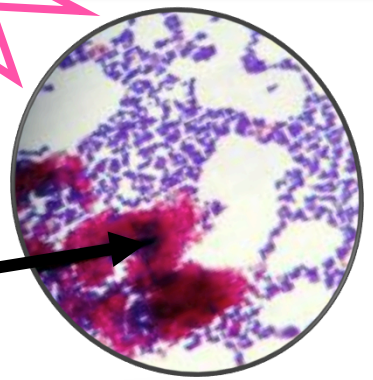
Q: Why Gram variable?

- Both **leprosy** and **tuberculosis** caused by *M. leprae* and *M. tuberculosis* respectively, have plagued mankind for centuries.
- Thought that *M. tuberculosis* and *M. leprae* evolved from a soil bacterium that infected cows, then made jump to humans about the time of animal domestication, 10,000 years ago.
- *M. tuberculosis* doubles population every 18-24 hours,
- *M. leprae* doubles population about every 14 days.

Q: What might be the impact of generation time on the course of the infectious diseases these microbes cause?



Acid-fast stain

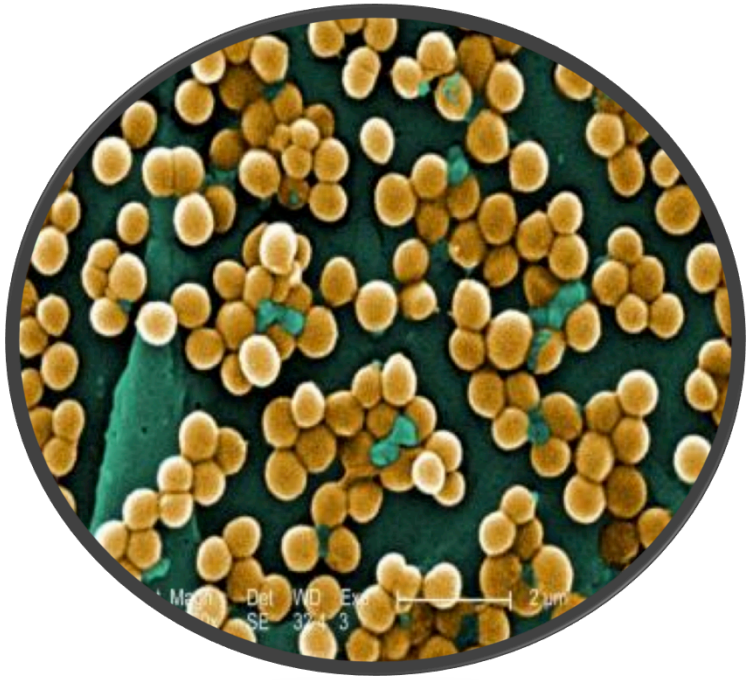


The pink is our lab friend *Mycobacterium smegmatis*

Images: TB Culture, Public Health Image Library (PHIL) #4428, Dr. George Kubica; 24 yo man from Norway, suffering from leprosy; Pierre Arents; Acid fast stain of *Mycobacteria smegmatis* & *Staph*, T. Port

Factors Influencing Microbial Growth

- Nutrition
- Oxygen
- Temperature
- pH
- Osmotic Pressure



This scanning electron micrograph (SEM) reveals numerous clumps of methicillin-resistant ***Staphylococcus aureus*** bacteria, commonly referred to by the acronym, MRSA, by Janice Haney Carr, PHIL #10046

Microbial Nutrition

- Organisms use a variety of **nutrients** for:
  - their energy needs
  - to build organic molecules & cellular structures.
- Most common nutrients contain necessary elements:
  - Carbon
  - Oxygen
  - Nitrogen
  - Hydrogen
- These 4 elements make up 95% of dry weight of bacterium.
- The other 5% is composed of Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus and Iron.
- Other elements that are needed are **trace elements**.
- These elements are needed in extremely small amounts, can be obtained through water intake.

Image: Salmonella, Rocky Mountain Labs NIAID NIH

Microbes & Oxygen

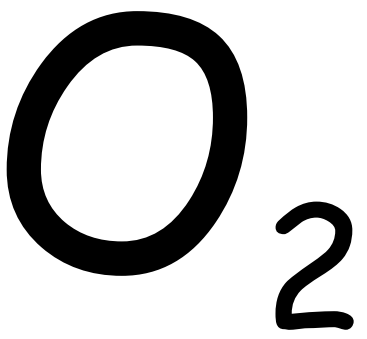
- **Obligate Aerobes** – Need oxygen to stay alive.
  Aerobic respiration = Use of O2 to break down food into useable energy.

- **Obligate Anaerobes** – Die in presence of oxygen. It is poisonous to them.
  Anaerobic respiration = break down food into useable energy without the use of O2.

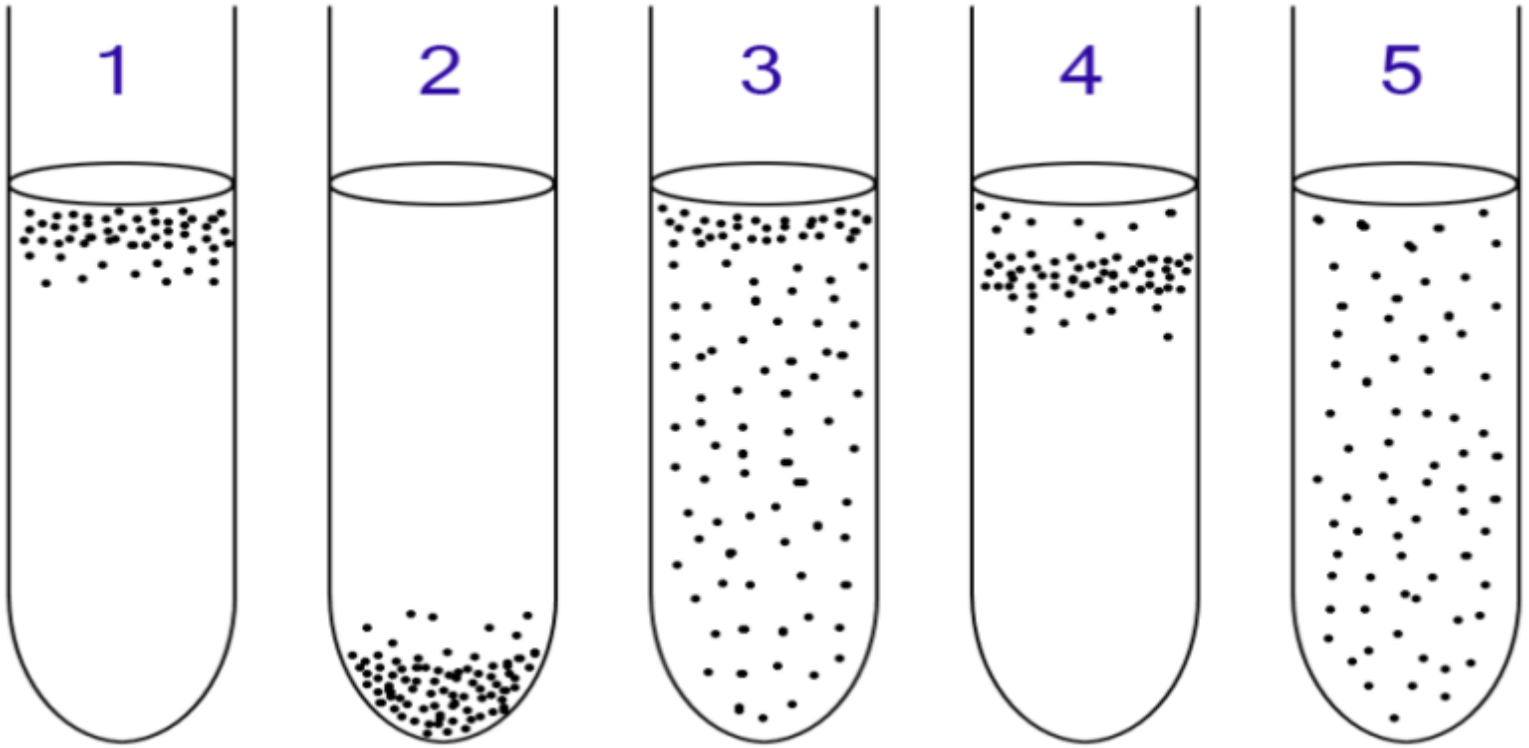
- **Facultative Anaerobes** – Not strict aerobes or anaerobes.
  Many yeasts and enteric bacteria. Escherichia coli and *Staphylococcus aureus.*

- **Microaerophilic bacteria** – Require oxygen levels lower that that found under normal atmospheric conditions (*Helicobacter pilori – found in stomach).*

- **Aerotolerant Anaerobes** – Don't use oxygen, but are not killed by it.
  (Lactobacilli - This genus will make pickles from cucumbers and cheese from milk.)



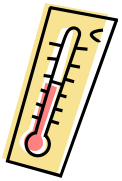
Microbes & Oxygen



Aerobic and anaerobic bacteria can be identified by growing them in liquid culture:

1: **Obligate aerobic** bacteria gather at top of test tube to absorb maximal amount of oxygen.
2: **Obligate anaerobic** bacteria gather at bottom to avoid oxygen.
3: **Facultative anaerobes** gather mostly at the top, since aerobic respiration is most beneficial; but as lack of oxygen does not hurt them, they can be found all along the test tube.
4: **Microaerophiles** gather at upper part of test tube, not at top. Require O_2, but at low concentration.
5: **Aerotolerant** bacteria are not affected by oxygen, and they are evenly spread along the test tube.

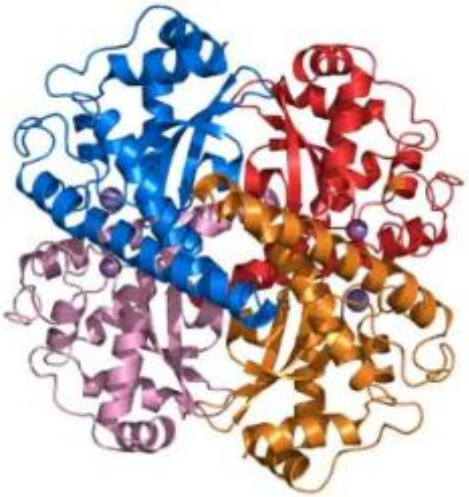
Image: Microbial oxygen requirements, Pixie



Microbes & Temperature

Proteins

- Three-dimensional shape because of the temperature sensitive hydrogen bonds.
- These bonds will usually break at higher temperatures, and protein becomes **denatured**.
- Denatured proteins lose function.



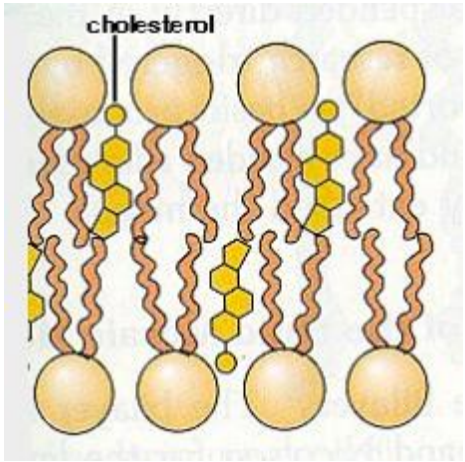
Lipids

Also temperature sensitive.

Become ***brittle*** if temperature is too low.

If temperature too high, lipids will be more ***liquid*** in form.

Outside membrane cannot preserve the integrity of the cell and it will disintegrate.



Images: Superoxide dismutase enzyme structure, Fvasconcellos; Phospholipids & Cholesterol, Cytochemistry.net

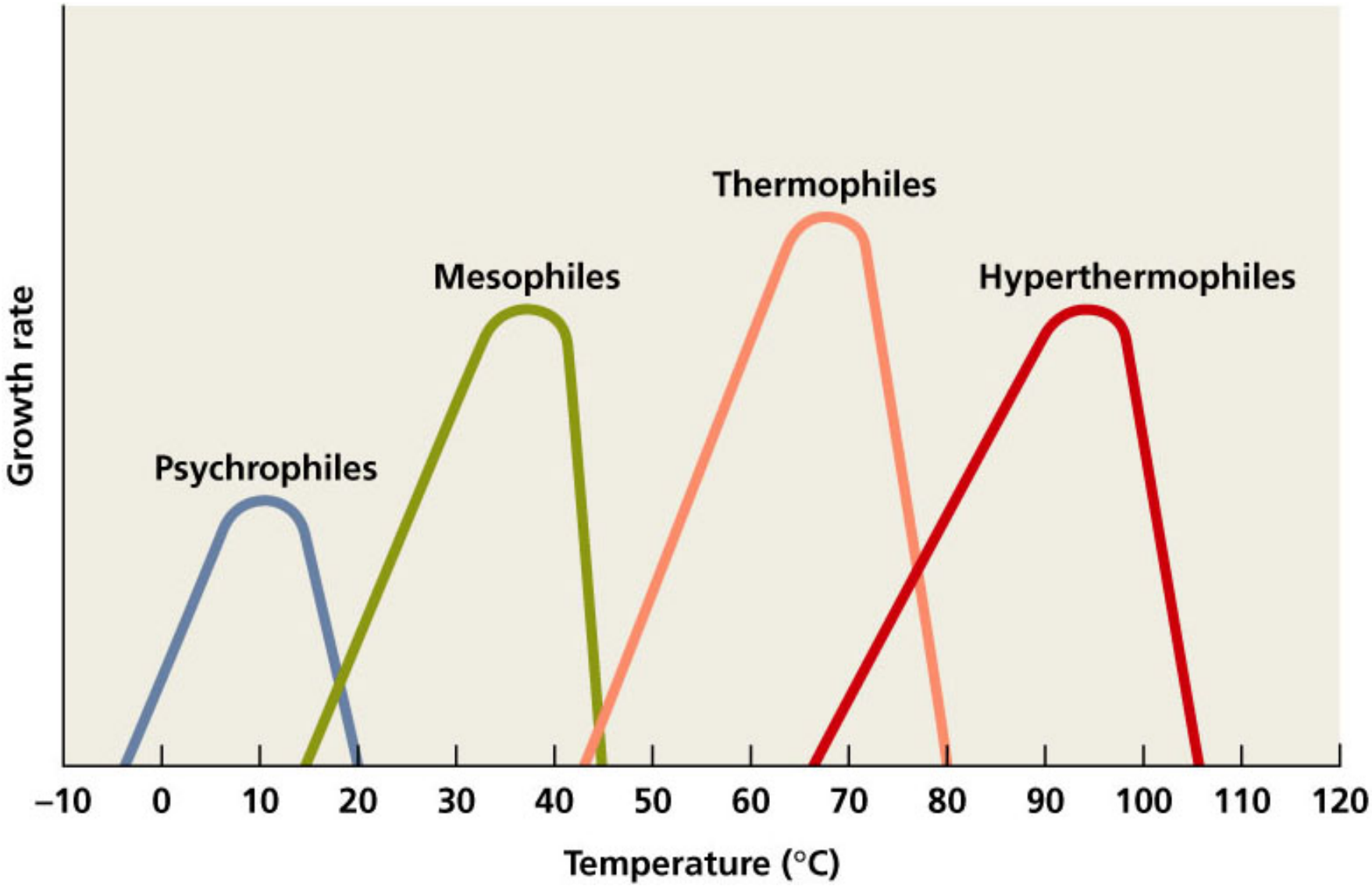
Effects of Temperature on Growth

5°C	25°C	35°C
40°F	77°F	95°F

Most of our plates are incubated at 37°C (98.6°F).

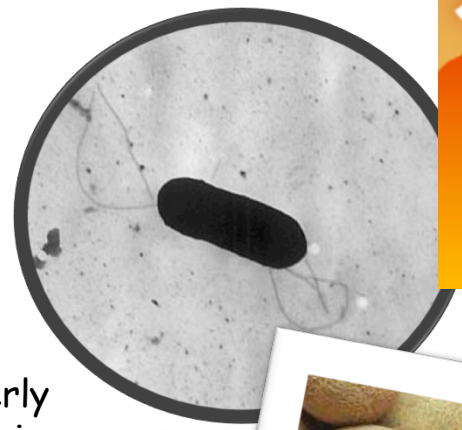
Conversion C to F = 1.8 x C + 32

Categories of Microbes Based on Temperature Range



Meet the Microbe! *Listeria monocytogenes*

Gram positive, rod-shaped **psychrophile**.



- *L. monocytogenes* is widely distributed; found in soil, water, animals, birds, insects.
- Responsible for disease listeriosis.
- Rarely pathogenic in healthy adults (mild flu-like symptoms).
- Can be lethal in pregnant women, fetuses, newborns, elderly and immune compromised, causing meningitis or bacteremia.
- Transmitted from environment (contaminated food & water) to human, except in the case of pregnant woman passing on to fetus.
- In vulnerable populations can have a case fatality rate of 25%.
- Facultative intracellular pathogen. Triggers its own phagocytosis.
- *Listeria* are very hardy. Can grow in temperatures ranging from 39°F (refrigerator) to 99°F.

***Q**: What microbes have we discussed in previous lecture that are at the other end of the temperature spectrum?*

CDC Investigation Announcement:

As of October 6, 2011, a total of 109 persons infected with outbreak-associated strains of *Listeria monocytogenes* have been reported from 24 states. All illnesses started on or after July 31, 2011.

Twenty-one deaths have been reported: One woman pregnant at the time of illness had a miscarriage.

Image: *Listeria monocytogenes*, PHIL #2287, Cantaloupe, USDA photo by Scott Bauer. Image Number K7355-11

Microbes & pH

As with temperature, bacteria have minimum, optimum and maximum pH ranges.

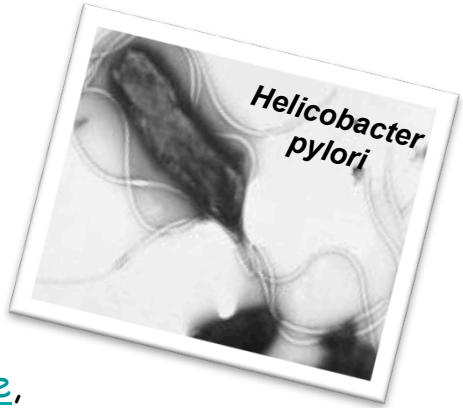
Neutrophiles

- **Protozoans and most bacteria** have an optimum pH range of 6.5 to 7.5.
- pH range of human organs and tissues.

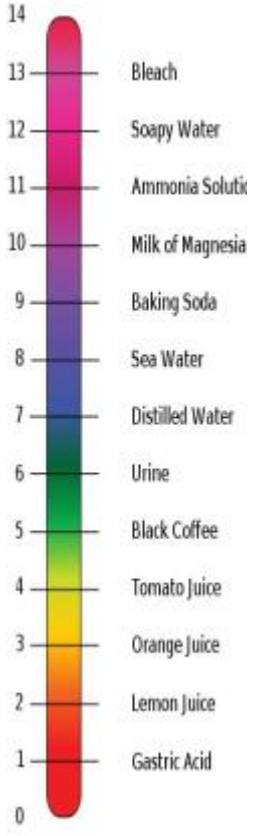
Acidophiles

- **Most fungi & some bacteria** grow best in acid niches.
- ***Obligate acidophiles*** have to live in an acidic environment.
- ***Acid-tolerant Microbes*** will survive in an acid environment, but do not prefer that.

Meet the Microbe!



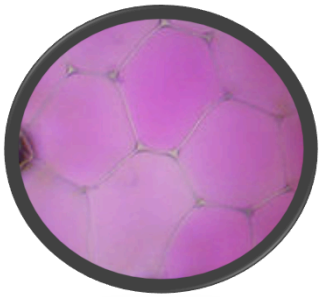
- Gram-negative, microaerophilic, and acidophilic bacterium.
- Can thrive in the stomach and upper small intestines and cause ulcers.
- However, many who are infected do not show any symptoms.
- *Helicobacter spp.* only known microorganisms to thrive in highly acidic environment of stomach.

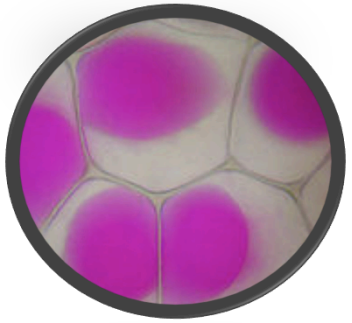


Images: *Helicobacter pylori*, Y. Tsutsumi, M.D., Fujita Health University School of Medicine; pH scale, Edward Stevens

From the Virtual Microbiology Classroom on ScienceProfOnline.com

Microbes & Water: Osmotic Pressure





- H_2O important reactant in many metabolic reactions.
- Most cells die in absence of water.
  - Some have cell walls that retain water.

    Q: *What genus comes to mind?*
  - Endospores and cysts can cease most metabolic activity for years.

    Q: *What organisms make endospores? Which make cysts?*
- Cell walls of bacteria prevent them from exploding in a **hypotonic** environment, but most bacteria are vulnerable in **hypertonic** environments.
- Many bacteria can be plasmolyzed by high concentrations of solutes.
- The water moves out of the bacterium and it dies of 'hyperosmostic shock' (desiccation).

Q: *Why can you keep honey on the cupboard for months, even years, without it spoiling?*

Images: Water drop, Fir0002, flagstaffotos.com.au; : Cells, full of water versus plasmolyzed, Mnolf, Wiki

Glycocalyx & Osmotic Pressure

Obligate Halophiles

- Must live in a niche of high salt content.
- Can grow in an environment up to 30% salt.
- If placed within a freshwater environment, they will burst and die.

Facultative Halophiles

- Can survive and tolerate high salt niches, but do not require them to living.

Some bacteria have an additional layer outside of the cell wall called the **glycocalyx**.

One type of glycocalyx is called a **slime layer**.

- glycoproteins loosely associated with the cell wall.
- cause bacteria to adhere to solid surfaces and help prevent the cell from drying out

Meet the Microbe!

The slime layer of *Staphylococcus* allows it to exist on the salty environment of the skin.

Photo: Sweat on face of runner; Bibikoff; Mannitol salt agar, T. Port

From the Virtual Microbiology Classroom on ScienceProfOnline.com

Confused?

Here are links to fun resources that further explain cellular respiration:

- Microbial Growth & Metabolism **Main Page** on the Virtual Cell Biology Classroom of Science Prof Online.
- Cellular Respiration animation by Jay Phelan, "What is Life? A Guide to Biology", W. H. Freeman & Co.
- Anaerobic Respiration Page by Timothy Paustain, University of Wisconsin, Madison.
- Electron Transport Chain animation from Molecular & Cellular Biology Learning Center.
- Food Molecules video from HowStuffWorks, a Discovery company.
- "The Energy" song by Audiovent.
- **Diffusion, Osmosis & Active Transport** Main Page, Virtual Cell Biology Classroom of Science Prof Online website.
- Bacterial growth video and narration, YouTube, Dizzo95..
- "The Osmosis Song" music video by Duanie Films.

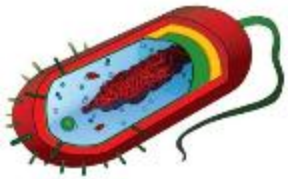
(You must be in PPT slideshow view to click on links.)

Are microbes intimidating you?

Do yourself a favor. Use the…

Virtual Microbiology Classroom *(VMC)* !

The VMC is full of resources to help you succeed, including:



- practice test questions
- review questions
- study guides and learning objectives

You can access the VMC by going to the Science Prof Online website
www.ScienceProfOnline.com

Images:, *Staphylococcus,* Giant Microbes; Prokaryotic cell, Mariana Ruiz