* Microbiology: the study of microorganisms
  + Divided into the three domains of life (*Archaea, Bacteria, and Eukarya*)
    - Archaea:
      * Distinguished from Bacteria by rRNA sequences and a lack of peptidoglycan
    - Bacteria:
      * Usually single-celled
      * Cell walls contain peptidoglycan
    - Eukarya:
      * Contains a nucleus
      * Includes protists, fungi, plants, and animals
    - Viruses: acellular entities that must invade a host cell in order to replicate
* Cellular Morphology:
  + Mycelium: branched network (with or without crosswalls)
    - Composed of *hyphae*
    - Illustrates the problem of pleiomorphic organisms (organisms that have variable shape)
      * Can lead to improper classification without a phylogenetic context (see DNA notes later)
* History of Microbiology:
  + Early days:
    - Leuwenhoek first to see microorganisms through a microscope
      * Four types of flagella (would have seen if he had stains):
        + Monotrichous: singular flagella
        + Lophotricious: multiple flagella emerging from one side of the microorganism
        + Amphitrichous: flagella emerging from both poles of the microorganism
        + Peritrichous: multiple flagella emerging from all areas of the microorganism
  + Spontaneous generation: theory that living organisms could arise from nonliving matter
    - Championed by John Needham
    - Pasteur and Tyndall experiments put this idea down
      * Tyndallization: sterilization of media via several rounds of boiling
  + Golden Age of Microbiology:
    - Microorganism-disease link recognized
    - Need for immunity/sterilization procedures realized by Lister
    - Agar and petri dish developed for bacterial culture by Koch and assistants
    - Pasteur developed anthrax and rabies vaccines (helped lead to discovery of antibodies and role of cellular immunity), yeast fermentation, and Pasteurization
    - Fleming discovers penicillin
    - Winogradsky discovers bacteria oxidize nitrogen and sulfur
    - Beijernick discovers bacteria fix nitrogen from the environment for use in energetic processes
  + Koch’s Postulates:
    - 1) The microorganism must be present in every case of the disease but absent from healthy organisms.
    - 2) The suspected microorganisms must be isolated and grown in a pure culture.
    - 3) The same disease must result from injection of isolated microorganism into a healthy host.
    - 4) The same microorganism must be isolated again from the diseased host.
  + Golden Age taxonomy:
    - Based on morphology and physiology (cell shape/size, energy sources, etc.)
      * Led to arbitrary-seeming phylogenies (phenotypes are often *pleomorphic* and therefore not stable within a species or closely related species)
* Bacterial Taxonomy and Phylogenetics:
  + Taxonomy: description and classification of organisms
    - Developed by Linnaeus
  + Phylogenetics: evolutionary relationships among species
    - Provides framework for species have certain traits and how they may change in the future
  + Modern Development:
    - Mayr introduced the biological species concept (a species is a group of organisms capable of producing fertile offspring)
      * Didn’t help much with microbiology (asexual reproduction)
    - Species concept in bacteriology: a species is a collection of organisms sharing many stable characteristics over time
      * Known as *polyphasic taxonomy*
      * Features examined include morphology, % G+C content, immunoreactivity, phospholipid profiles, and genetic exchange
      * Genomic DNA reassociation of > 70% sometimes used as a distinguisher
    - DNA sequencing returned phylogenetic context to microorganismal taxonomy
      * Genetic fingerprints and DNA barcode regions began to be used to distinguish species
      * Better because phenotypes are subject to convergent evolution (dissimilar species independently evolve similar phenotypes due to the environment)
    - Bacteriological Species Definition: (current def.) a species is a *monophyletic* group of organisms that share many stable characteristics and differ from other organisms
      * Monophyletic: traced to a single common ancestor with all organisms of the group descending from it (CANNOT EMPHASIZE ENOUGH HOW IMPORTANT THIS IS TO REALLY GET)
      * Ribosomal phylogeny: all organisms have ribosomes with sequences descended from a common ancestor
        + Different regions change/evolve at different rates
  + Bergey’s Manual of Systemic Bacteriology is used today as the foremost source for bacterial and archaeal taxonomy
    - Strains: collection(s) of organisms that are of the same species but differ in some criterion
    - Isolates: individual microbial organism derived from a single colony
    - OTU: operational taxonomic unit; used for differing kinds of isolates
* Origin of Cellular Life:
  + 1) formation of organic precursor molecules
    - Urey-Miller experiment demonstrated abiotic formation of organic compounds can occur with an energy source
      * Possible energy sources include lightning, meteorite strike/shockwave, evaporative lagoons, and geothermal vents
      * Liposome: lipid vesicle
      * Probiont: liposome and RNA complex (with or without proteins)
  + 2) formation of biopolymers
  + 3) assembly into cells with replicating membranes and intact cellular biology
    - Oldest fossilized cells are about 3.5 billion years old (may be bacterial or archaeal)
    - Morphological and chemical evidence includes stromatolite formation, microfossils, 13C PDB depletion (depletion shows life exists because of its preferential use by life), etc.
* Origin of Eukarya:
  + Genome similarities:
    - Metabolic genes and organelles more similar to Bacteria
    - DNA replication, transcription, and translation genes more similar to Archaea
  + Genome fusion theory: archaeal and bacterial cells fused and certain functions from each domain were incorporated into Eukarya
  + Endosymbiotic theory: mitochondria and chloroplasts were prokaryotic organisms that fused with early eukaryotic cells and developed a symbiotic relationship
* *Escherichia coli (E. coli):*
  + Discovered by Theodore Escherich
  + Lives in the large intestine
    - May assist in food digestion
    - Also exists in mucous membrane linings overtop *villi*
      * Disease symptoms caused if bacteria bind to the villi
    - Natural environment is wet and rich in organic matter
      * High throughput of material
        + Peristalsis: rhythmic contractions forcing food particles and other materials through the digestive tract
        + Epithelial cell desquamation: replacement of cells in the epithelial tissue
    - Intestinal microbial community contains around 1012 organisms
      * *E. coli* is the most prevalent facultative anaerobe (can grow with or without oxygen) in the intestines
        + Is excreted in feces
  + Intestinal infections by *E. coli*
    - Can be dirrheagenic by six different pathotypes (mechanism of disease)
    - Factors infection depends upon:
      * Agent in question
      * Virulence:
        + Agent in question falls under this category
      * Exposure method
      * Dose
        + Exposure and dose can be combined into the category of *transmission*
        + ID50 graphs can depict the amount of dose per unit time needed to achieve a certain chance of infection for a particular strain
      * Susceptibility
        + Related to genetic variation in humans
        + Affected by age, weight, habits, and overall health
        + Also affected by the body’s *resident microbes*
    - *E. coli* most often infects the body via food
    - Virulence factors: organism characteristics contributing to pathogenicity
      * Adherence to surface tissue(s)
      * Avoidance of host defenses
      * Toxin production
        + Endotoxin: toxin released upon bacterial death
        + Exotoxin: toxin secreted during bacterial growth
      * Invasiveness to tissues other than those at the point of infection
* *E. coli* pathotypes:
  + ETEC: enterotoxinogenic *E. coli*
    - Transmissible from person to person
    - Two types of toxin:
      * ST (stable): stays stable in the presence of heat
      * LT (labile): unstable in the presence of heat
    - Globally important; can contaminate water
  + EIEC: enteroinvasive *E. coli*
    - Invades the actual epithelial cells
    - not as virulent as ETEC
    - evolving toward *Shigella*
      * *E. coli* may contain the entire Shigella genus according to phylogenetic evidence
  + EHEC: enterohemmorhagic *E. coli*
    - Is a microvilli-effacing pathogen
    - Can cause outbreaks in the United States
    - Can cause hemorrhagic diarrhea, hemolytic urine syndrome, kidney failure, and death
    - Spreads via food (Chipotle) 🡪 *E. coli O:157-H7*
    - STEC: Shiga toxin strain of EHEC bacteria
  + EPEC: enteropathogenic *E. coli*
    - Is a microvilli-effacing pathogen (changes the actin filaments)
    - Transmissible from person to person
    - Water contaminant and globally important (historically in U.S.); kids especially vulnerable to EPEC
  + EAggEC: aggregative *E. coli*
  + DAEC: diffusely aggregating *E. coli*
  + ExPEC: extraintestinal pathogenic *E. coli*
    - Often causes urinary tract infections and meningitis
    - Often hospital-acquired (so-called nosocomial infection)
    - Common in the U.S.
* Environmental Factors Changing Microbial Growth:
  + Reproduction is via binary fission
    - Septum formation: formation of a new cell membrane in between two dividing microbial cells
      * Triggered by attainment of a threshold length, mass, and completion of DNA replication
      * FtsZ protein: protein forming a contractile ring at the septum in order to pinch off the two dividing cells
        + Utilizes GTP
    - Fastidious organisms: organisms that cannot synthesize all needed biomolecules and must acquire some from the environment
  + Three growth factors:
    - Amino acids
    - Purines and pyrimidines
    - Vitamins
  + Ways to measure size/growth rate:
    - Direct microscopic counts
    - Growth to visible colonies (number of CFUs: colony forming units)
    - Turbidity and absorbance
    - Biomarkers (ex. Quantity of DNA, ATP, PLFA, cell wall constituents, taxon-specific sequences)
  + “Normal” Microbial Conditions:
    - 15-40 degrees C
    - 20% O2
    - pH 5.0-5.8
    - atmospheric pressure
    - < 0.64 M salinity
  + Terminology Describing Different Species:
    - X-phile: organism that must grow under alternate condition X
    - X-tolerant/X-troph: organism that can grow under alternate condition X
* Temperature and Microbial Growth:
  + Terminology describing X-temperature lovers
    - Psychrophiles: organisms capable of growth and development at extremely cold temperatures
    - Psychrotrophs: organisms capable of survival (and possibly growth) at extremely cold temperatures
    - Mesophiles: organisms growing best at moderate temperatures
      * *E. coli* is a mesophile
    - Thermophiles: organisms capable of thriving at relatively high temperatures
    - Hyperthermophiles: organisms capable of thriving at extremely high temperatures
  + Effects on Enzymes:
    - Rule of thumb is that the rate of chemical reactions doubles every increase in temperature by 10 degrees Celsius
  + Membrane Fluidity:
    - Lower temperatures decrease membrane fluidity
      * Problem can be solved by adding more unsaturated fats to increase kinks in the membrane
    - Higher temperatures increase membrane fluidity
      * Too high temps can disintegrate membrane (problem solved by adding more saturated fats to membrane)
* pH, Salt Concentration, and Pressure:
  + pH terms:
    - Acidophiles: organisms thriving at pH’s in between 0-5.5
    - Alkalophiles: organisms thriving at pH’s in between 8.5-11
  + Salt terms:
    - Halotolerant: organisms that can survive at salt concentrations of 0-3M
    - Halophiles: organisms that can thrive at salt concentrations of > 0.7M
  + Pressure:
    - Barophiles: organisms capable of surviving at extreme (above atmospheric) pressures
  + *E. coli* is not adapted to these extremes
* Oxygen and its Toxic Effects:
  + Toxic O2 derivatives:
    - Super oxide radical (O2-)
    - Hydrogen peroxide
    - Hydroxyl radical (OH)
  + Enzymes Dealing w/Toxic Derivatives:
    - Superoxide dismutase: enzyme dealing with super oxide radical (SOD abbrev.)
    - Catalase: enzyme catalyzing decomposition of hydrogen peroxide to water and oxygen
    - Peroxidase: enzyme catalyzing oxidation of hydrogen peroxide
  + Oxygen Tolerance and Organisms:
    - Obligate aerobe: organism that requires oxygen to survive (utilizes SOD and catalase)
    - Facultative aerobe: organism capable of using oxygen (utilized SOD and catalase)
      * *E. coli* is a facultative aerobe
    - Aerotolerant anaerobe: organism not utilizing oxygen but capable of surviving in its presence (utilizes SOD)
    - Strict anaerobe: organism not capable of surviving in the presence of oxygen
    - Microaerophile: organism capable of surviving at extremely low levels of oxygen (utilizes SOD and low levels of catalase)
* *E. coli* environment:
  + Why doesn’t it grow well elsewhere?
    - Mouth has a larger flow of cells and material
    - Stomach pH is too low
    - Skin is low in moisture and high in salt, acidic, and has antimicrobial secretions
    - Genitourinary tract is acidic, high urea concentrations, and a high throughput of material
    - Immune system protects other areas
  + Why doesn’t it grow in other habitats?
    - Soil and water have lower nutrient concentrations and more complex organics
      * Desiccation: drying out due to low moisture
      * Lower temperature
      * Altered adhesion sites
    - Has a half-life of ten days in the environment
      * Movement occurs with water
      * Used as an indicator for fecal contamination
* *E. coli* structure:
  + Rod-shaped with peritrichous (several randomly distributed) flagella
  + Has an outer cell membrane, a periplasmic space, peptidoglycan, and a plasma membrane that is Gram-negative
    - Plasma membrane: innermost layer of the outer cellular structure composed of a phospholipid bilayer
      * *Phospholipid profiles of fatty acid chains can be used for bacterial identification!!!!!*
      * Selectively permeable and houses metabolic functions using a chemical gradient
    - Peptidoglycan/murein: two alternating amino-monosaccharides found in the periplasmic space
      * NAG: n-acetylglucosamine
      * NAM: n-acetylmuramic acid
        + Four peptide chains attached to each NAM

Has some D-configuration amino acids (L found in most proteins)

DAP: diaminopimetic acid; unique amino acid found in NAM

* + - * Chains are helical and can crosslink in any direction
        + Provides stability to structure
    - Periplasmic space: liquid/gel-filled space between the plasma and outer membranes
      * Thicker in Gram-negative bacteria
    - Outer cell membrane: lipopolysaccharides forming the outermost layer of *E. coli*
      * Attached to peptidoglycan via *Braun’s lipoprotein*
      * Lipopolysaccharide composition:
        + Negatively charged
        + O-side chain/O-antigen: portion of the lipoprotein causing the immune response

Can be changed by bacteria to avoid the response

Causes a systemic inflammatory response (is an endotoxin)

* + - * Proteins present:
        + Integral proteins are not easily removed (have a transmembrane motif)

Are also *amphipathic*

* + - * + Peripheral proteins are more easily removed (reach into the periplasmic space)
  + Inside *E. coli* structure:
    - Inclusion bodies: aggregations of proteins, carbohydrates, organic acid polymers, some lipid and protein shells
      * May not actually be found in *E. coli*
    - Nucleoid: dense area in the cell where the chromosome is located
      * Not membrane-bound
    - Bacterial chromosome is circular and tightly packed
      * Has around 4.6 megabases
    - Plasmids: small, circular pieces of DNA separate from the chromosome
      * May contain important genes for antibiotic resistance, toxin production, and pilus formation
      * Used in genetic engineering
  + Structure outside the cell wall:
    - Several types of protein appendages:
      * Fimbriae/Adhesion pili: hairlike protein tubes extending from the cell
        + Helps *E. coli* adhere to surfaces (usually epithelial tissue) and increases motility

Protein called adhesin mediating binding at the tip

* + - * + Important virulence factor for ExPEC, ETEC, EPEC, and EHEC strains
      * Sex pili: thicker protein tubes extending from the cell
        + Allows plasmid DNA to be transferred from one cell to another (*conjugation*)
      * Flagella: taillike protein tubes used for motility
        + *E. coli* have *peritrichous flagella* (several and randomly distributed)
        + Anchored to the cell wall via a basal body

Basal body also rotates *rod and ring proteins* attached to the main flagellum filament

Driven by ion gradients (does NOT use ATP)

* + - * + Filament is composed of *flagellin* protein units

Sometimes coated in a sheath (but not in *E. coli*) for protection

* + - Other structures:
      * Slime layers: layers of polysaccharides and protein outside the cell wall
        + Diffuse, unorganized, and easy to remove
        + Used for attachment to surfaces and protection from predators, toxins, pH fluctuations, and desiccation
        + Combined slime layers from many cells are called a biofilm

Provides extra protection for the bacteria inside

Bacteria form their own microenvironment

* + - * Capsule: layers of polysaccharides and protein outside the wall that is organized and not easily removed
        + A special type of capsule or slime layer composed only of polysaccharides is called the *glycocalyx*
        + Same functions as slime layers
        + Important virulence factor for ExPEC strains (also O-antigens)
* Growth and Systems:
  + Types of lab growth media:
    - Complex: supplies a large assortment of growth factors
    - Defined/synthetic: only has individually added growth factors
  + Rates:
    - Continues as long as resources are replenished, wastes are removed, and a portion of the population is removed
  + Systems:
    - Open: system in which nutrients are replenished, waste removed, and resources maintained at constant levels
    - Closed: system in which some environmental factor is restricted so as to determine the effect on growth
  + Phases:
    - Lag phase: intracellular synthesis of materials needed for growth
    - Exponential growth: phase in which growth rate is limited only by the resources in lowest supply relative to need
    - Stationary phase: phase in which resources are depleted and population growth stagnant
    - Death phase: period of reduction of population size
    - Dormant phase: phase in which spores and dormant cells are formed to prolong lifespan in the face of depleted resources