* 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