Dynamics of Prokaryotic Growth
Chapter 4

Prokaryotic Growth Conditions

- Wide range
  - From deep oceans to volcanoes
  - From polar regions to equator
  - From Great Salt Lake to fresh-water streams
- Each species different
  - May grow at boiling point, but not below
  - May grow in Great Salt Lake, but not fresh-water streams

Pure Cultures

- _____ pure cultures in nature
  - ____________ species in communities
- Why do we want pure cultures in lab?
  - ____________ ____________ of particular species
  - Helps us determine ____________ ____________ of disease

Pure Cultures

- Population of organisms descended from a ____________ ____________
  - Separated from other species
- ___ 1% of all prokaryotes can be cultured in lab
  - Includes most medically important species
- Special techniques to achieve culture success
  - Sterility
    - Glassware, media, instruments
  - Aseptic techniques
    - Minimize contamination

Growing Bacteria on Solid Media

- Bacteria form ____________ on solid medium
- Colony
  - Mass of cells all ____________ from original cell
  - Cannot see with naked eye until about a million _______ cells present
Chapter 4 Dynamics of Prokaryotic Growth

Growing Cultures

- Liquid (__________)
  - Contains ideal nutrients for growth
  - _______ isolate pure cultures in _________
- Solid
  - Liquid with ________, a gelling agent
- Agar
  - Complex polysaccharide derived from marine _________
  - Add more to make media more solid
- Containers
  - Petri dish (“___________”)
  - ________ (“deeps”; “slants”)

Characteristics of Agar

- ________ to bacterial degradation
- Survives ________ temperature treatment
  - Can be sterilized in autoclave
- ________ at ________ temperatures
  - Can be poured into convenient containers
- Stays liquid until below ______ °C
- After cooling, ________ ________ over wide temperature range
  - Until above 95°C
- ________
  - Allows colonies to be seen

Streak Plate Method for Isolating Pure Cultures

Stock Cultures of Pure Cultures

- ________ pure culture
- Take ________ of colony
- ________ onto agar medium in tube
  - “Agar ________”
- Why slants?
  - Larger surface area
  - Condensation?
- Maintain as inoculum for later study
Chapter 4 Dynamics of Prokaryotic Growth

Understanding Bacterial Growth

- Growth
  - Increase in number of ______________ in a population
- Binary fission
  - Cell increases in size
  - Doubles contents, including DNA
  - Divides in two
  - Growth is ______________
    - $1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow 32 \rightarrow 64$

Generation Time

- ______________ time = ______________ time
- Depends on ______________ ______________
  - e.g. Temperature
  - e.g. Nutrient levels
- Depends on ______________
  - *Escherichia coli* – 20 minutes
  - *Mycobacterium tuberculosis* – 12-24 hours
- Exponential growth means pathogens can “explode” in numbers in short time
  - e.g. potato salad example in book:
    - 10 cells $\rightarrow \sim 40,000$ cells in 4 hours!

Exponential Growth Calculations

- $N_t = N_0 \times 2^n$
- $N_t$ = number of cells in a population at some time of interest (“$t$”)
- $N_0$ = original number of cells in the population (at time “0”)
- $n$ = number of generations in the time elapsed
- Potato salad example
  - $N_0 = 10$ cells
  - $t = 4$ hours
  - Generation time = 20 minutes (3 generations per hour)
  - $n = (3 \times 4) = 12$
  - $N_t = 10 \times 2^{12} = 40,960$
Chapter 4 Dynamics of Prokaryotic Growth

Factors Affecting Microbial Growth
- Temperature
- Oxygen
- pH
- Water availability

Environmental Factors Affecting Microbial Growth
- Range: ~25°C between upper and lower limits
- Optimum somewhere in range
  - reactions temperature specific
    - Speed of reaction increases for each ______ °C
    - Can be ________ if temperature gets too high
    - Some have proteins with _______ - _______ 2° & 3° bonds
- Optimum temperature may be used as classification method

Classification Based on Temperature Optima
- (-5°C – +15°C)
  - e.g. Listeria (food poisoning)
  - Prefer > 15°C, but tolerate lower
  - e.g. Pseudomonas spp.
- (25°C – 45°C)
  - e.g. Escherichia coli
  - Most other common bacteria
- (45°C – 70°C)
  - Lactobacillus delbrueckii ssp. bulgaricus (yogurt)
- (70°C – 110°C)
  - Members of Archaea
    - Pyrolobus fumarimii isolated from hydrothermal vent has max growth temp of 113°C

Growth Rates at Various Temperatures
- Why do some prokaryotes tolerate high temperature?
- Enzymes (proteins) from thermophiles and hyperthermophiles do _______ denature with heat
  - Denaturation is function of sequence of amino acids
    - Sequence determines bonds in three-dimensional structure
Chapter 4 Dynamics of Prokaryotic Growth

Importance of Temperature

- Food ________________
- Spoilage organisms usually retarded by cold
  - Refrigeration is ~ ____ °C
  - Psychrophiles and ________________ can still grow
- Increased temperatures often mean food poisoning organisms can grow

Temperature effects on disease ________________

- Some parts of body are lower in temperature
  - ________________ – feet, hands, nose, ears, fingers
- Some organisms can only grow in cooler regions of body
  - *Mycobacterium leprae* (leprosy) grows better in extremities
  - Syphilis lesions on genitalia, lips, tongue, throat
    - ________________ were induced to treat syphilis using malaria before antibiotics

Oxygen

- Different species ________________ to different levels of oxygen
- Ecological ________________
  - ________________ O₂
    - e.g. surface of skin
  - ________________ or ________________ O₂
    - e.g. soil, water, parts of body
  - ________________ Low or ____ O₂
    - e.g. intestines, stomach, swamps
- Some organisms killed or inhibited by O₂

  ________________ Derivatives of O₂

- Produced during normal metabolism or as reaction with light
  - ________________ (O₂⁻)
  - Hydrogen ________________ (H₂O₂)
- Survival in O₂ requires special enzymes to detoxify
  - ________________ ________________ (SOD)
    - O₂⁻ → H₂O₂
  - ________________
    - H₂O₂ → H₂O + O₂
Chapter 4 Dynamics of Prokaryotic Growth

Determining $O_2$ Requirements

- Grow unknown in “_____________ tube”
- _______________ tube of nutrient agar
  - _______________ _______________ $O_2$ & _______________ agar
- _______________ to 50°C
- Add organism
  - _______________ or swirl
- Cool and incubate at appropriate temperature
- $O_2$ in medium _______________ by solidified agar
- Organism grows at _______________ $O_2$ level

Classification by Oxygen Requirements

- Obligate aerobes
- Facultative anaerobes
- Obligate anaerobes
- Microaerophiles
- Aerotolerant anaerobes

Classification by Oxygen Requirements

- _______________ aerobes
  - _______________ $O_2$ for aerobic respiration
  - Cannot grow/multiply without $O_2$
    - e.g. *Pseudomonas spp.*
- Obligate _______________
  - _______________ reproduce in presence of $O_2$
  - Pervasive in environment
  - ½ of all cytoplasm on earth!!!!
- No adaptation to toxic derivatives
  - e.g. *Bacteroides spp.* – large intestine
  - e.g. *Clostridium spp.* – soil organisms
  - C. botulinum – botulism toxin
Chapter 4 Dynamics of Prokaryotic Growth

Classification by Oxygen Requirements

- _______________ anaerobes
  - Grow _______________ in _______________ of O₂, but can grow without
  - Use _______________ respiration if O₂ available
  - More ATP → faster growth
  - _______________ or _______________ respiration if unavailable
    - e.g. *Escherichia coli*
    - e.g. *Saccharomyces cerevisiae* (yeast – eukaryote)

Classification by Oxygen Requirements

- _______________
  - Require _______________ amounts of O₂
    - Over 2-10% O₂ inhibitory
    - e.g. *Spirillum volutans* aquatic environments
    - e.g. *Helicobacter pylori* – gastrointestinal ulcers
  - _______________ anaerobes
    - _______________ to level of O₂
    - Grow in presence of O₂ but do _______ respire aerobically
    - “_____________ fermenters”
      - e.g. *Lactobacillus bulgaricus* – cheese and yogurt
      - e.g. *Streptococcus pyogenes* – “strep throat”

Environmental Factors Affecting Microbial Growth – pH

- Most can live between pH ______ and pH ______
- May have adaptations to maintain internal neutrality
  - Even though externally extreme
- _______________
  - Optimum of pH 7 – most bacteria
  - May have adaptations to neutralize local environments
    - e.g. *Helicobacter pylori* makes urease
      - Splits urea in stomach → CO₂ + ammonia → neutralizes local area
- _______________
  - Optimum of < pH 5.5
    - e.g. *Thiobacillus ferroxidans* – best at pH 2.0
      - Oxidizes sulfur for energy → sulfuric acid as by-product
Chapter 4 Dynamics of Prokaryotic Growth

- Pumps out excess protons to keep internal pH neutral

- Optimum of > pH 8.5
- e.g. *Bacillus alcalophilus* – best at pH 10.5
- Antiporter exchanges ions to maintain internal neutrality

**Environmental Factors Affecting Growth – Water Availability**

- availability affected by substances
  - e.g. NaCl or sugars water availability
  - If solute concentration in environment higher than in cell → water cell
    - Cell dehydrates
    - Membrane
    - “” if able to tolerate up to 10% NaCl
    - e.g. *Staphylococcus spp.* on skin
    - “” if able to tolerate even higher NaCl concentrations
  - What happens if solute concentration outside is lower than inside cell?

**Adjusting to Excess Osmolarity in Environment**

- Pump ions out of cell to maintain internal osmolarity e.g. K⁺
- Produce small amino acids, to equalize osmolarity e.g. proline

**Importance of Osmolarity and Water Availability**

- Reduced → reduced
  - In most cases
  - dissolved salts or sugars to environment → bacterial growth
  - Food preservation
    - Examples of foods preserved using salt?
    - Examples of foods preserved using sugar?

**Nutritional Factors Affecting Microbial Growth**

- All cell products made from building blocks
  - Lipids
  - Carbohydrates
  - Amino Acids / Proteins
  - Nucleic Acids
- These compounds all formed from
  - Carbon
  - Hydrogen
  - Oxygen
  - Nitrogen
    - Prokaryotes only use N₂
Chapter 4 Dynamics of Prokaryotic Growth

Minor Elements, Trace Elements

- ____________ Elements
  - Needed in smaller quantities than major elements
    - Sulfur
    - Phosphorus
    - Calcium
    - Magnesium
    - Potassium
    - Iron

- ____________ Elements
  - Needed in very small quantities
    - Cobalt
    - Zinc
    - Copper
    - Molybdenum
    - Manganese

___________ Factors

- Low molecular weight compounds
  - ____________
  - ____________
  - ____________
  - ____________

“___________organisms” need ____________ growth factors supplemented

- e.g. Neisseria
- e.g. Lactobacillus spp.
  - Used for bioassays of vitamin levels
- May require unusual compounds
  - e.g. naphthalene (mothballs)!!
  - e.g. herbicides
  - e.g. plastics
  - e.g. Bacillus fastidiosus
    - Can use only urea and derivatives for both carbon and energy

Diversity in Energy Source and Carbon Source

- ____________
  - Phototrophs vs chemotrophs
- ____________
  - Autotrophs vs heterotrophs
Chapter 4 Dynamics of Prokaryotic Growth

The Autotrophs

- ________________ – “Primary Producers”
  - Use ___________ for energy
  - Use ___________ in atmosphere for carbon source to make organic molecules
  - Many other organisms rely on these in atmosphere
    - e.g. Cyanobacteria
    - e.g. Algae

- ________________ (“chemoautotrophs”)
  - Use ___________ compounds for energy (e.g. H₂S)
  - Use ___________ for carbon source
  - May live in inhospitable environments
    - e.g. hot sulfur springs
    - Primary producers for their environments

The Heterotrophs

- ________________
  - Use ___________ for energy
  - Carbon from ________________ carbon compounds
    - e.g. purple nonsulfur bacteria
      - Grow anaerobically using light for energy
      - Grow aerobically in dark, using organic sources of energy

- ________________
  - Use ___________ sources of ___________ and ___________
  - Most common among those associated with humans & animals
  - Beneficial and pathogenic groups
  - Varies how fastidious species are

Bacterial Growth Under Laboratory Conditions

- Open systems
  - Nutrients added, waste removed
  - ___________ growth
- Closed systems different from open systems
  - Tubes of broth
  - Plates of agar

- Nutrients are ___________
  - May run out
  - ___________ for resources occurs
  - Results in characteristic growth stages: Growth Curve
Dynamics of Prokaryotic Growth
Chapter 4

Growth Curves

- Four distinct stages
  - Lag phase
  - Exponential (Log) phase
  - Stationery phase
  - Death phase

__________________________ Phase
- ________________ for cell division – ______ cell division
  - Synthesis of macromolecules
- Length of phase depends on conditions
  - Richness of medium
  - Stored in refrigerator then suddenly transferred
  - Age of cells

__________________________, or Exponential Phase
- ________________ growth
  - ________________ cell division
  - Generation time ________________ here
  - More ________________ to antibiotics and other chemicals
- ________________ log phase
  - ________________
  - ________________
  - Growth materials or waste materials
- ________________ log phase
  - ________________ metabolites produced

__________________________ Phase
- Resources ________________ or ________________ accumulated
- Total number viable cells remains relatively ________________
- Death = cell division
- ________________ stationary phase
  - ________________ metabolites may still be produced – see below
- Cells more resistant to antibiotics, chemicals, and radiation
Chapter 4 Dynamics of Prokaryotic Growth

__________ Phase

- __________ death
- Death of cells __________ cell division
- Cells die at __________ rate
- Usually not as steep a slope as the growth phase

Colony Growth

- Growth in colonies follows growth curve
- Different parts of colony at __________ __________ of growth curve
- Resources depleted sooner in __________ of colony
  - __________ between cells in colony restricts growth
  - __________ metabolites accumulate more quickly in center of colony
- Exponential growth continues at __________
- Plane of division or enzyme production at colony edges may produce intricate patterns

Intricate Patterns of Growth by Some Colonies

- Patterns may vary from species to species
- Colony morphology may change when same species is grown on different media

Bacterial Growth in Nature

- __________ in pure culture
- Nutrients may be replenished
- Cells may be __________ by other organisms or swept away by currents
- Sometimes develop strategies to adhere to surfaces or to interact with other species

__________ Microbial __________

- In nature, species live in close association with other species
  - e.g. mouth, intestines, soil, water
- Interactions between species optimize __________ for other species
  - e.g. aerobes use up O\textsubscript{2}, reducing O\textsubscript{2} levels for anaerobes
  - e.g. metabolic wastes may provide nutrients for another species
- Difficult to reproduce in laboratory
  - May be hard to grow these species
Chapter 4 Dynamics of Prokaryotic Growth

Biofilms

- Bacteria attached to surfaces encased in polysaccharide (___________)
  - Slippery rocks in streams
  - Slime in kitchen drain
  - Scum in toilet bowls
- Plaque on teeth
- Slime on cooling towers
- Can you think of others???

___________ Formation

- Bacterium ____________ to surface
- Produces loose ____________
- Other ____________ may attach to glycocalyx and grow
- Cells in characteristic architecture with open channels for nutrients and waste
- ____________ - ____________ communication important in structure

Biofilms in Medicine and Environment

- Serious problem in
  - ____________ to antibiotics or other chemicals
  - ____________ decay
  - ____________ disease
  - Other bacterial infections
- May be beneficial in
  - ____________
    - Improves ability of bacteria to degrade chemicals such as oil or pesticides

Methods of ____________ and ____________ Bacterial Growth

- Direct Cell Counts
  - Counts ______ cells – alive or dead
- Viable Cell Counts
  - Counts only those able to ____________
    - Good for monitoring growth in food or water
- Measuring ____________
  - Measures turbidity, total weight, or nitrogen
- Measuring Cell ____________
  - Measure by-products of metabolism
Chapter 4 Dynamics of Prokaryotic Growth

Methods of Detecting and Measuring – Direct Cell Counts

- _______________ microscopic counts – rapid
  - Counting chambers on special slides
  - Hold measured volume of liquid
  - Grid facilitates counts
  - Must be ≥ _____ cells/ml for accuracy

Methods of Detecting and Measuring – Direct Cell Counts

- Cell counting instruments
  - _______________ Counter
    - Cells in suspension of saline
    - Measures changes in resistance past aperture
    - Cells less conducting
  - _______________ _______________
    - Measures change in laser light scatter past aperture
    - May tag some cells with fluorescent dye to differentiate types

Methods of Detecting and Measuring – _______________ Cell Counts

- Quantifies number capable of multiplying

Two approaches:

- Actual counts of colony-forming units (______________)
  - Plate counts
  - Membrane filtration

- Statistical _______________
  - Most probable number (MPN)

Methods of Detecting and Measuring – Viable Cell Counts

- Actual counts – ________________ counts
  - Isolated cell on agar plate grows to single colony
  - Dilute culture to find countable dilution
  - Use dilutions at 10-fold concentration differences
  - Count only plates with 30 – 300 colonies
  - One colony = one ________________ cell in original sample
    - Colony-forming unit (______________)

4 - 14
Chapter 4 Dynamics of Prokaryotic Growth

Methods of Detecting and Measuring – Viable Cell Counts

- Plate counts
  - ____________-plate method
    - 0.1 – 1.0 ml dilution into empty sterile Petri dish
    - Add melted nutrient agar cooled to 50°C
    - Cells trapped and grow in cooled agar
  - ____________-plate method
    - 0.1 – 0.2 ml dilution onto surface of solid nutrient agar
    - Spread with glass spreader

Methods of Detecting and Measuring – Viable Cell Counts

- Actual counts – ____________ ____________
  - Pass sample through fine-pore filter (sterile)
  - Known volume
  - Trap organisms on filter
  - ____________ filter to agar plate for growth
  - Count CFUs
  - Number of CFUs = cells/volume

Methods of Detecting and Measuring – Viable Cell Counts

- Statistical ____________
  - ____________ ____________ (MPN)
  - Serial dilutions in 10-fold increments
  - Replicated tubes of broth for each dilution
  - Incubate and assess growth
  - e.g. gas production
  - Compare with MPN table for estimate of cell numbers
  - Commonly used to determine coliforms in water samples
    - Gram-negative rods
    - Lactose-fermenters
    - Indicate fecal contamination

Methods of Detecting and Measuring – Viable Cell Counts

- MPN statistical method
Chapter 4 Dynamics of Prokaryotic Growth

Methods of Detecting and Measuring – Biomass

- __________
  - Cloudiness due to light scatter among cells in liquid
  - Requires high cell concentration to detect by eye
    - $10^6$ still clear
    - $10^7$ barely cloudy
  - Measure using ______________
  - Measures transmittance of light to detector
    - Inverse of optical density

Methods of Detecting and Measuring – Biomass

- Total __________
  - Good for filamentous organisms
    - Hard to count individual cells
  - Centrifuge for wet weight
    - Proportional to total number of cells
  - Dry at 100°C for 8-12 hrs
    - Dry weight = ~30% wet weight
    - Cell is approximately 70% water

Methods of Detecting and Measuring – Cell Products

- By-___________ of metabolism
  - ___________ production from breakdown of sugars
    - Use pH indicator in media
    - Color change indicates acid
  - ___________ production
    - Durham tubes trap gas bubbles in broth
    - Indicates fermentation w/ CO₂ production
  - ___________ production
    - Assessed using luciferase
    - Firefly enzyme
    - Light produced if viable organisms make ATP

Methods of Detecting and Measuring – Cell Products

- Gas trapped in Durham tubes