Bacterial Growth, Environmental Effects and Strategies

Microbial Physiology

Module 2

Aims and Objectives

- By the end of this modules you should…
  - Understand the processes of bacterial growth
  - Be able to describe the phases of bacterial growth
  - Be able to distinguish between methods of determining bacterial growth
  - Understand the effects of…
    - Nutrient levels
    - Temperature
    - Oxygen
    - Osmotic pressure
    - pH

      on bacterial growth

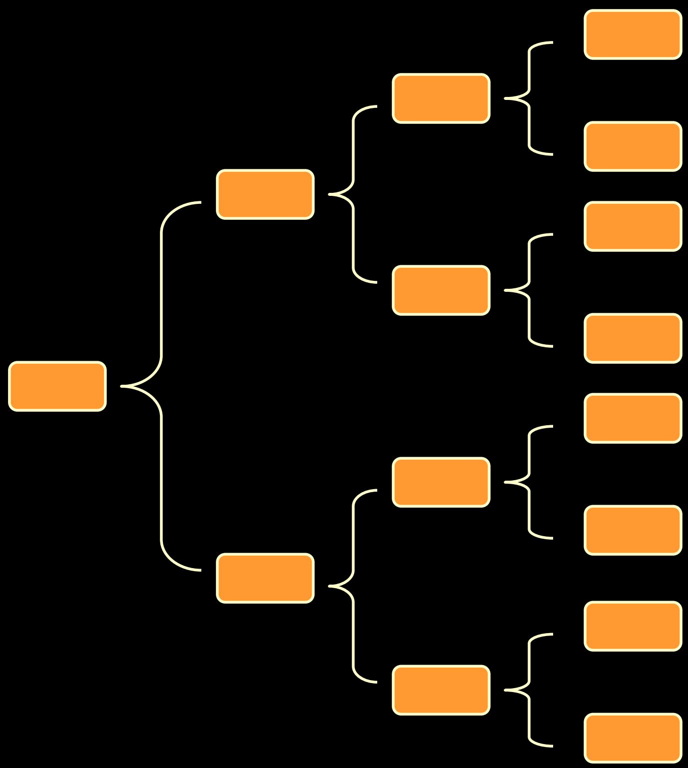
Bacterial Growth

- Bacterial growth equates to cell reproduction
  - Compare growth of multicellular vs unicellular organisms
    - Multicellular: increase in the size of the organisms
    - Unicellular: increase in the number of individuals in the population

- Changes in cell number are used to monitor bacterial growth

Reproduction of Bacterial Cells

- Binary fission
  - Assexual reproduction
  - A cell divides to produce two identical progeny cells



Binary Fission

- Binary fission involves 3 processes
  - Elongation
  - DNA replication
  - Cell division

- Compare this arrangement to the

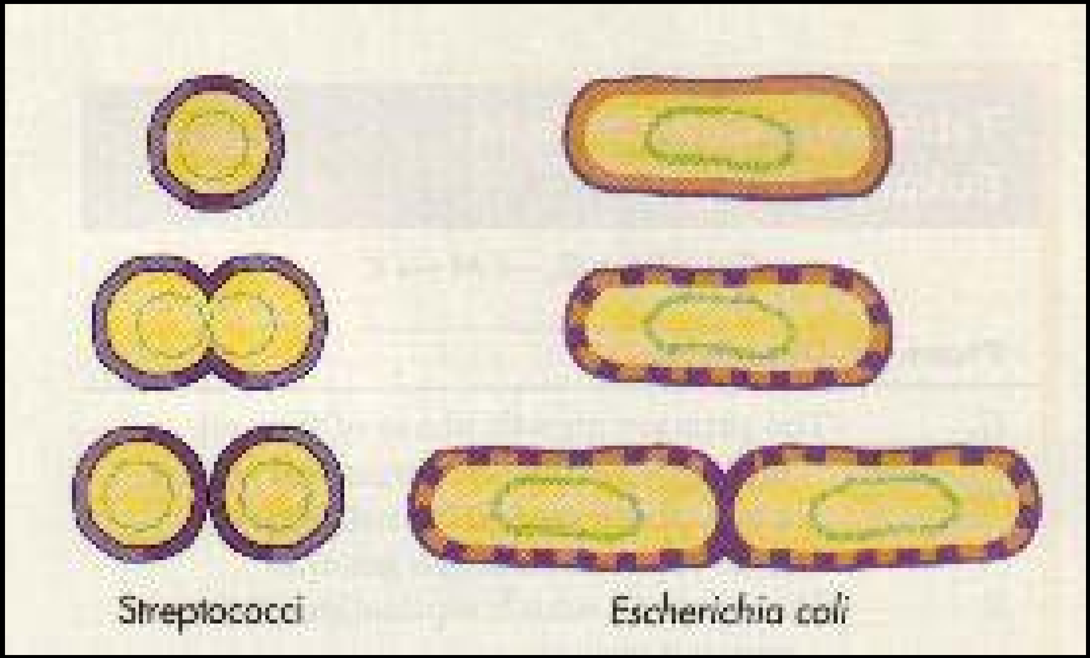
$$G_1 \rightarrow S \rightarrow G_2 \rightarrow M \rightarrow C$$

cell cycle in eukaryotic cells

Cell Elongation

- Biosynthesis of a new cell wall and membrane
  - Begins at specific sites located at the poles of cocci cells (eg. *Enterococcus*)
    - As new cell wall material is synthesised, is forced away from the site resulting in an elongated cell
  - For Gram-negative rods (such as *E. coli*)…
    - Cell wall is added all around the the cylindrical region
    - Outer membrane material is inserted at specific adhesion sites between the cytoplasmic and outer membrane

Cell Elongation Diagrams



DNA Replication

- For molecular details, see Module 1
- Duplication of the cell's chromosome
- *E. coli* cells reproduce every 20 minutes
- Replication of the *E. coli* chromosome takes 40 minutes
  - A new round of DNA replication is initiated prior to the completion of the previous round
  - Multiple replication forks
  - Dividing cells inherit one complete copy of the genome plus additional material produced as a result of subsequent replication

DNA Replication

- Chromosome duplication also offers a mechanism of regulation
  - DNA replication always begins at the origin
  - Genes closest to the origin are duplicated first
  - This effectively increases the copy number of these genes
    - Increases the products from these genes
  - Genes associated with cell wall / membrane synthesis are located near the origin

Septum Formation

- Partioning of chromosome and cytoplasmic components
- Formation of a crosswall between the two cells
- Chromosomes are separated by associations with the cytoplasmic membrane
- Septum is formed by invaginations of the cell membrane, followed by the cell wall

Growth Rate

- Time it takes a bacterial cell to reproduce
  - generation time or doubling time
  - denoted by "k"
    - reciprocal of the doubling time
    - see Atlas page 418 for full details
- is characteristic of bacterial species
- is defined by other factors
  - temperature
  - media conditions
  - pH

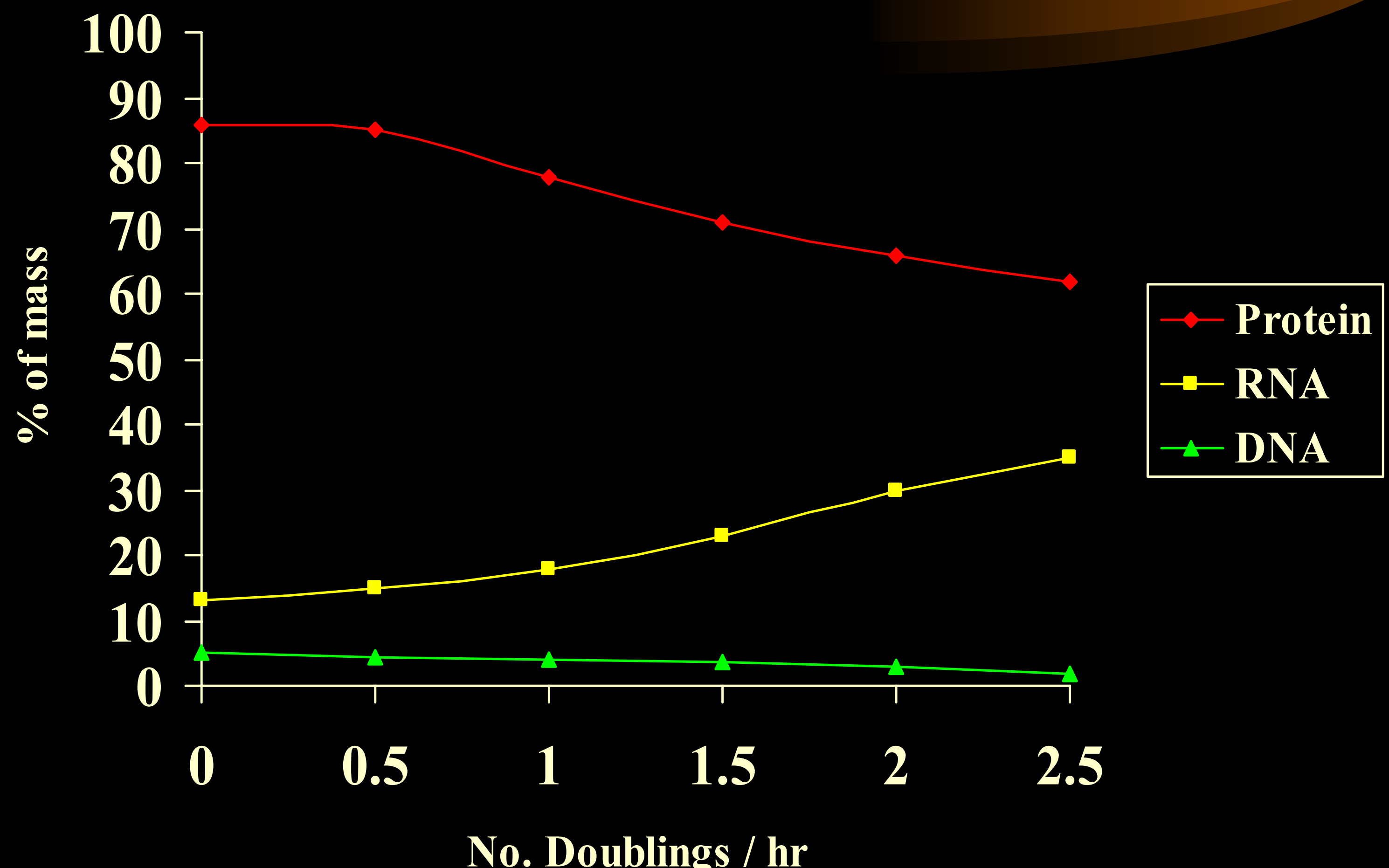
Growth Rate: Some Examples

Organism	Temp (ºC)	Generation Time (min)
Bacillus stearothermophilus	60	11
Escherichia coli	37	20
Pseudomonas putida	30	45
Vibrio marinus	15	80
Mycobacterium tuberculosis	37	360
Treponema pallidum	37	1980

Physiological Effects of Growth

- Cell mass increases
  - cells become larger during periods of rapid growth
  - increase in cell components is required
    - increase in DNA, RNA and protein
- There is a relative increase in RNA levels compared to DNA and protein at higher growth rates
  - due to increase in the number of ribosomes
  - as a result of cells requirement for more protein

Effects of Bacterial Growth on Macromolecular Composition of Cell



Genetic Adaptations for Increased Growth Rates

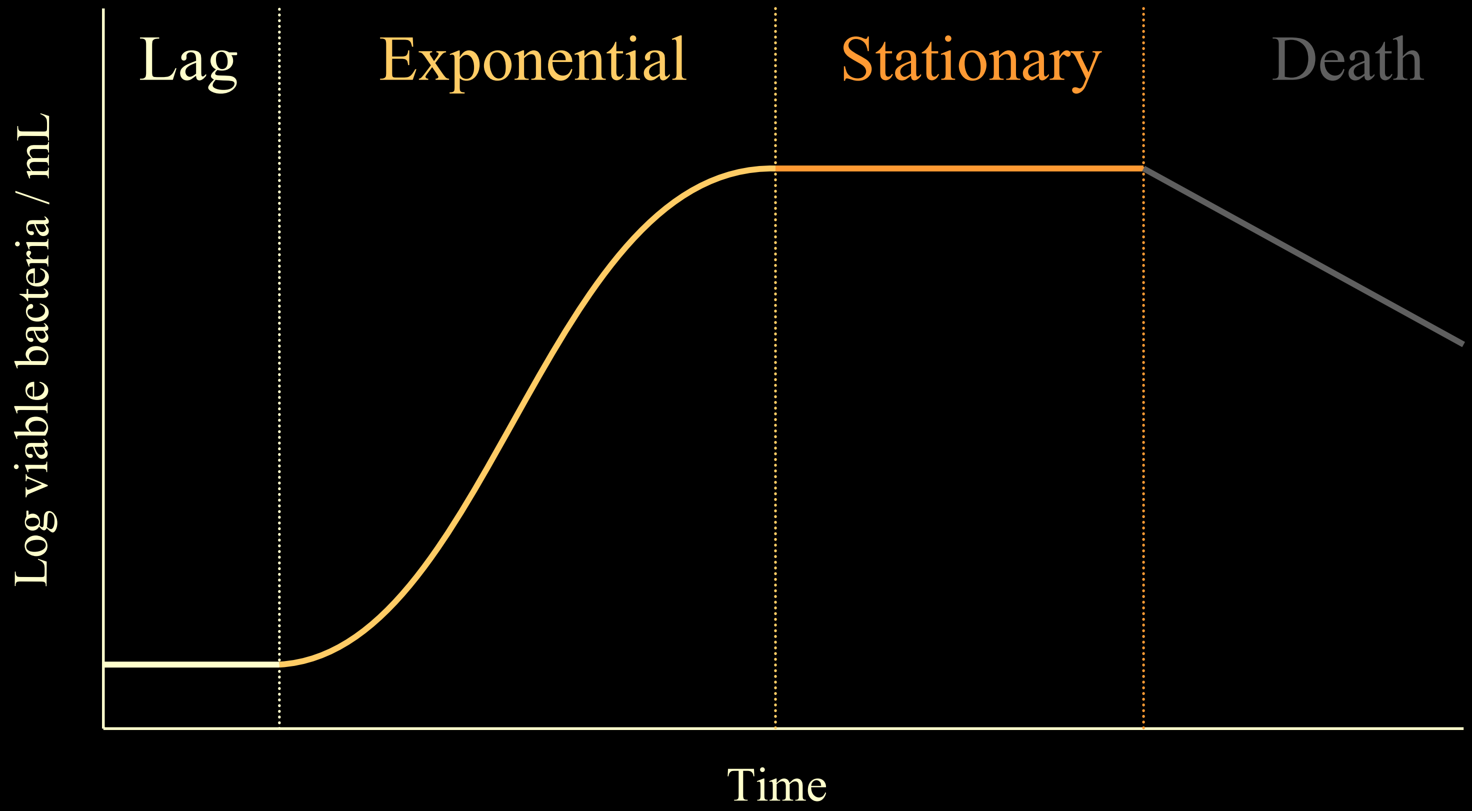
- Bacteria have evolved adaptations for increased gene expression for increased growth rates
  - in *E. coli*, genes encoding essential structures (eg OMP) are located near the origin of replication effectively increasing the dosage of these genes during replication
  - OMP mRNAs also have a longer half-life than mRNAs for genes involved in catabolism increasing the time they are available for translation

Population Growth Phases

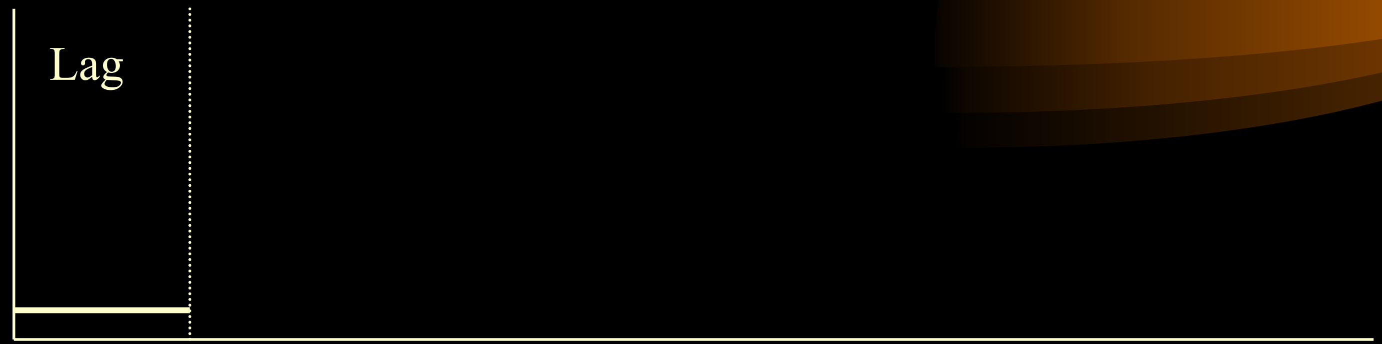
- Unrestricted growth
  - growth that occurs when there are no limiting factors on the population
    - eg. Nutrients
    - waste product accumulation
    - pH

- Balanced growth
  - synthesis of all cell constituents in a balanced manner

Bacterial Growth Curve

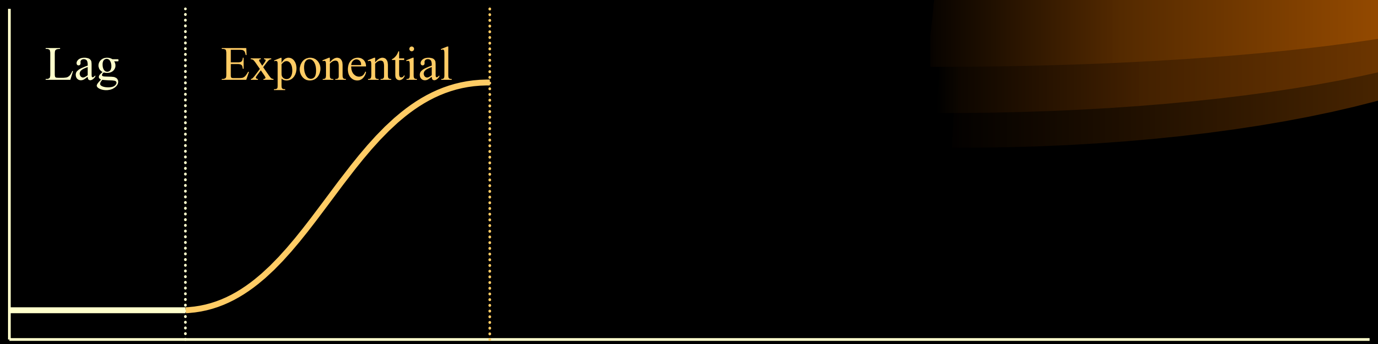


Lag Phase



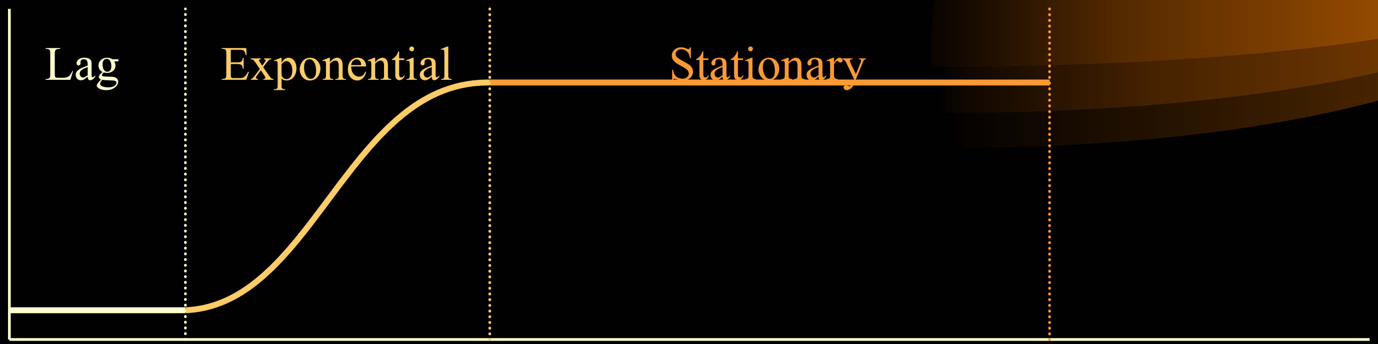
- Occurs when organisms are transferred to new medium
- Little increase in cell number
- Bacteria are transporting nutrients inside cell from the new medium
- Cells are preparing for replication and division
- Individual cells increase in size
- growth is generally unbalanced and unrestricted

Exponential (or log) Phase



- Bacterial cell division begins
  - proceeds as a geometric progression
  - cell numbers increase as an exponential function of time
- Growth is unrestricted, but balanced
  - the concentration of all macromolecules within the cells are increasing at the same rate

Stationary Phase

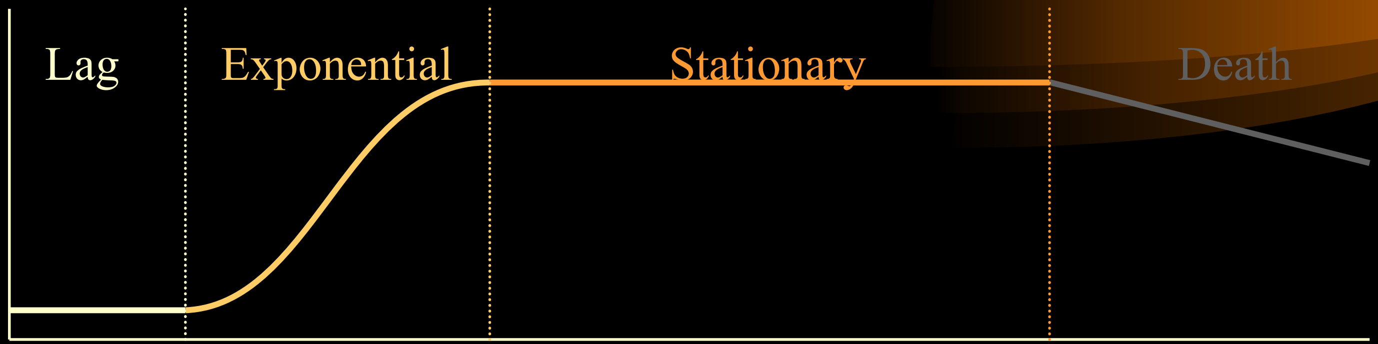


- No net increase in cell numbers
  - growth rate = death rate
- Brought about by exhaustion of nutrients, waste product buildup or changes in physical conditions
- Metabolic rate decreases
  - Feedback mechanisms regulate enzymes involved in key metabolic steps

Stationary Phase

- Cells are more resistant to environmental stresses
- Significant physiological changes can occur between cells in log phase and those in stationary phase
  - *eg. Arthobacter* cells change from rod-shaped cells (log phase) to cocci (stationary phase)
- Growth is unbalanced
  - various cellular components are synthesised disproportionately

Death Phase

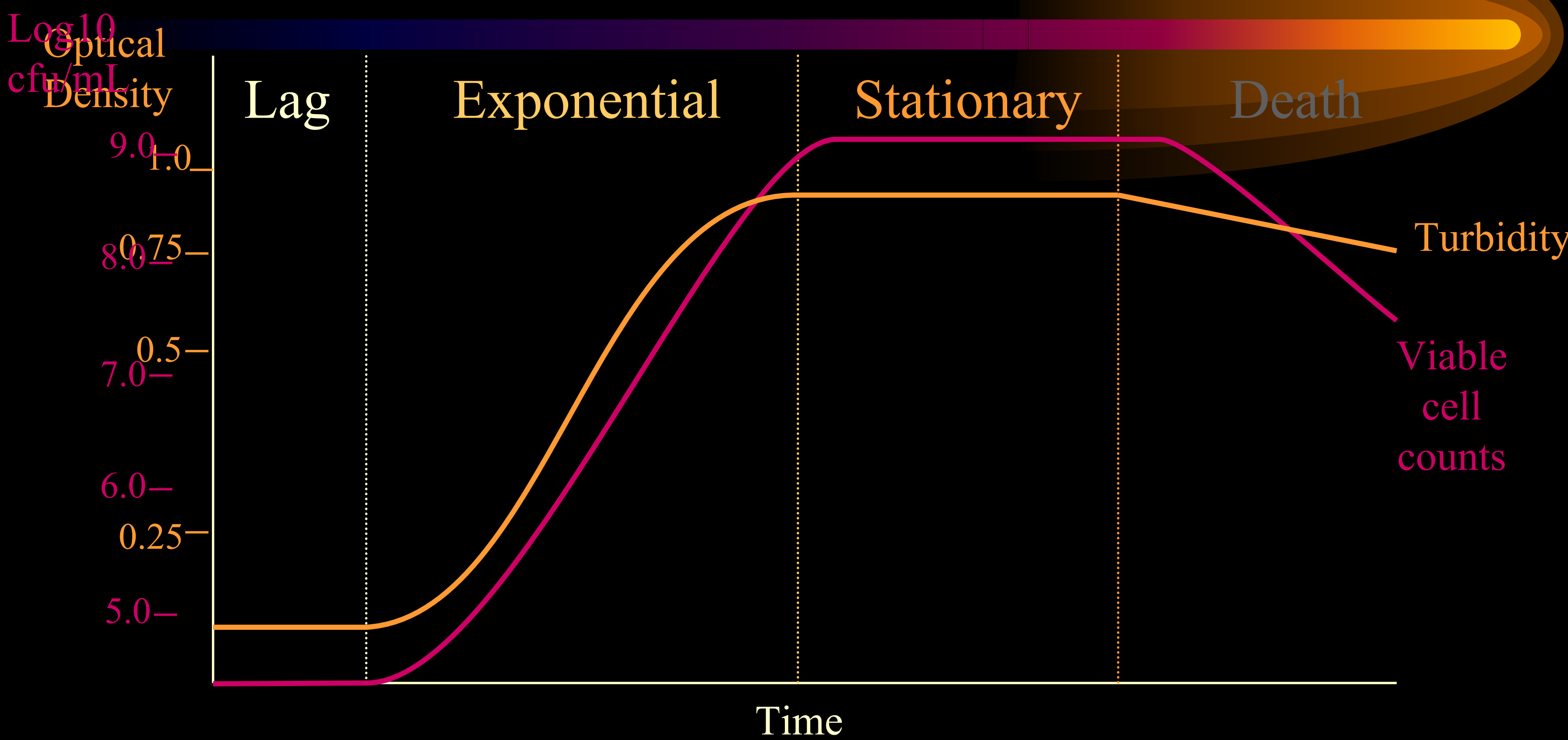


- Decline in cell numbers
- Brought about when toxins or waste products reach a threshold concentration
- Bacterial growth is restricted and unbalanced
  - cells cannot obtain all requirements for growth or replication

Measurement of Bacterial Growth

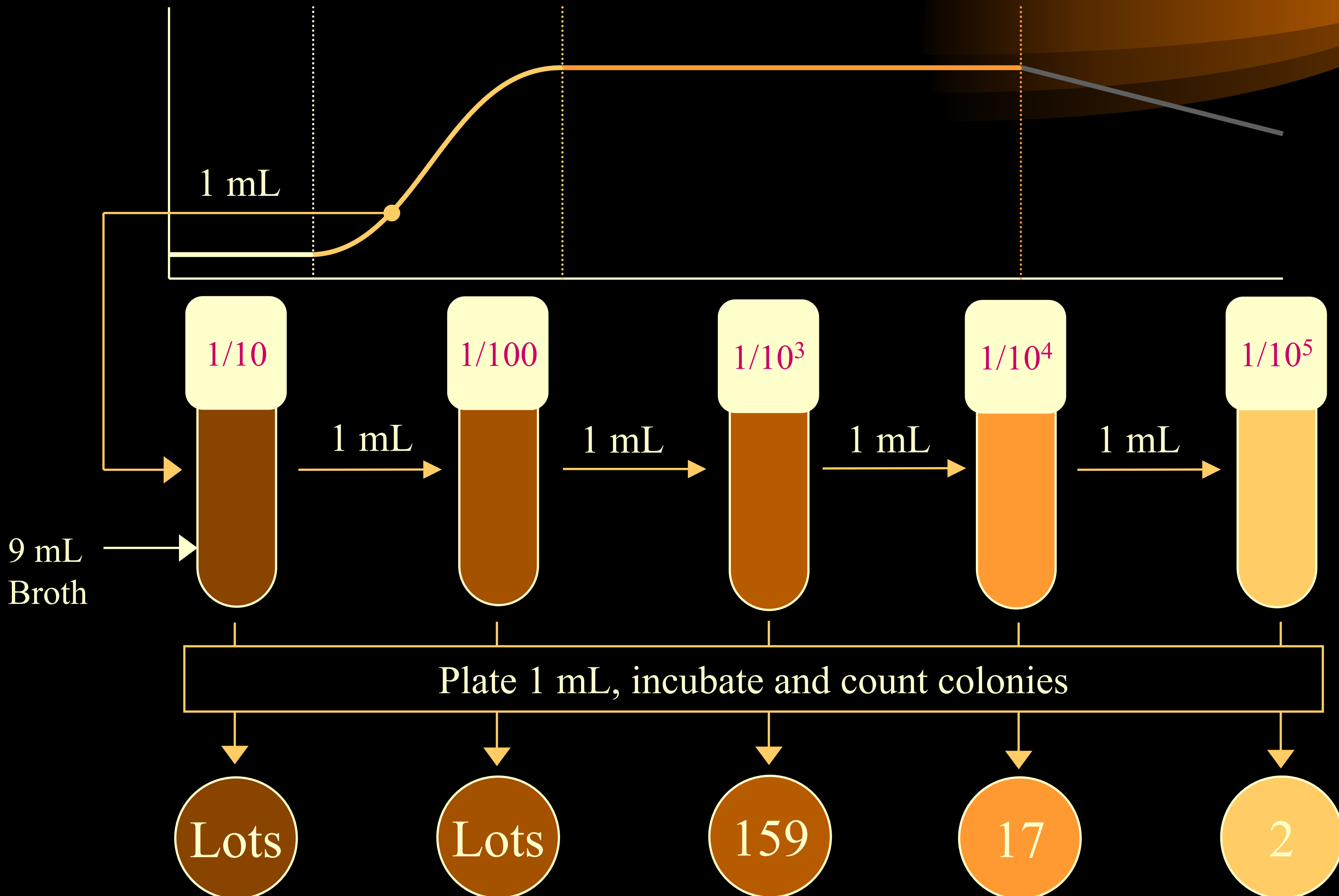
- Direct method
  - cell counts
    - either via a microscope or cell counter
- Indirect methods
  - colony counts (viable cell counts)
  - Spectrophotometry (turbidity)
  - Weight (dry vs wet)
  - ATP determination

Turbidity vs Viable Cell Count



- Death phase is less obvious when measuring turbidity as non-viable cells have similar absorption properties to viable cells

Viable Cell Counts



Growth of Bacterial Cultures

- In nature
  - conditions cannot be controlled
  - many species co-exist
  - changes in conditions may cause population shifts as conditions favour certain members of the population over others
- In the lab
  - conditions can be controlled
  - established to favour a particular organism
  - important in industrial processes for the accumulation of desired metabolic products

Batch Cultures

- produce bacterial growth curves just discussed
- inoculation of fresh media with bacteria
- nutrients are expended
- metabolic products accumulate
- closed environment
  - eg. Inoculating 100 mL of a rich media in a 1 L flask with *E. coli*

Continuous Culture

- Fresh medium replaces spent medium
  - continuous replenishing of nutrients and removal of waste products
  - permits continuous growth of culture
- Continuous culturing can be controlled by …
  - Turbidostat
    - monitors turbidity and cycles media as required
  - Chemostat
    - constant flow rate continuously cycles media
    - keeps cells in log phase

Synchronous Culture

- Synchronous growth
  - all cells divide at the same time
  - can be achieved by altering environmental conditions
    - repeatedly changing the temperature
    - adding fresh media to cells entering stationary phase
  - can only be maintained for a few generations

Effects of Nutrient Concentration on Bacterial Growth

- Nutrients
  - obtained from the environment
  - used for energy and biosynthesis of macromolecules
    - Cell solids components (H, O, C, N, P and S)
    - Cations (K, Mg, Ca, Fe, Mn, Co, Cu, Mo and Zn)
    - Anions (Cl)
    - Vitamins
- Most natural ecosystems are characterised by low nutrient levels
  - bacteria must be able to survive periods of starvation

General Strategies for Coping with Low Nutrient Levels

Starvation Factor	System	Genetic Control
Amino acids	Stringent response	*relA* (stringent factor) *spoT* (ppGpp degradation)
Ammonia	Ntr system	*glnA* (Glutamine synthetase) *glnG* (NR_I: response regulator) *glnL* (NR_{II}: histidine kinase)
Glucose	Catabolite repression	*cya* (adenylate cyclase) *crp* (catabolite repression protein)
Phosphate	Pho system	*phoBRUA*

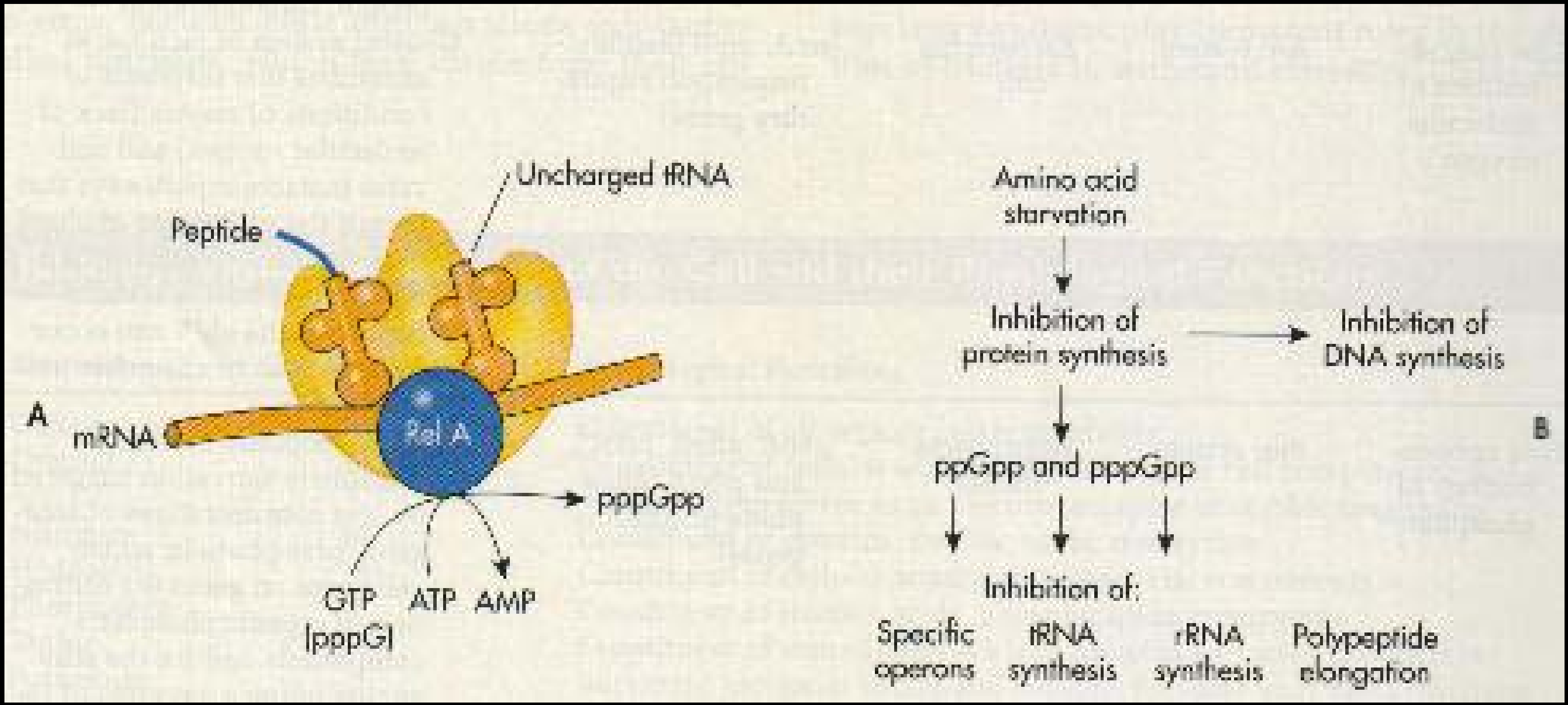
Stringent Response

- Response to depleted amino acid pool
  - amino acid starvation
- Mechanism for controlling the transcription of specific operons that code for rRNA and tRNA
- Reduces the rate of protein synthesis by decreasing the synthesis of rRNA
  - shuts down a number of energy-draining activities as a single response

How the Stringent Response Works

- Amino acid starvation results in the expression of *relA* (stringent factor)
- stringent factor associated ribosomes allow uncharged tRNA to bind to the A site
- stringent factor catalyses the pyrophosphorylation of GTP (to pppGpp) or GDP (to ppGpp)
- ppGpp may
  - inhibit transcription of tRNA and rRNA genes
  - cause stalling of translation and premature termination

Stringent Response



Ammonia Starvation

- Low levels of nitrogen
- Ntr System
  - scavenging system
- turns on genes for ammonia production from other nitrogen sources
- genes for glutamine synthetase are also induced
  - ATP-dependent assimilation of glutamine from low levels of ammonia
  - glutamine amino nitrogen can be transferred to other amino acids
  - ammonia supply for the cell

Ntr System in E. coli

- *glnA-glnL-glnG*
  - *glnA*: glutamine synthetase
  - *glnL*: NR_{II} (a histidine kinase)
  - *glnG*: NR_{I} (a response regulator)
- two-component regulatory system
  - discussed further in Module 4
  - phosphorylation of the histidine kinase
  - transfer of the phosphate to response regulator
  - response regulator acts as DNA binding protein and regulates transcription
- regulation of *gln* gene transcription is via σ^{54}

Phosphate Starvation

- Response to low levels of inorganic phosphate
- The Pho system
  - utilises phosphates from sources other than inorganic phosphates
  - involves over 100 proteins
  - over production of alkaline phosphatase
  - utilisation of phosphate from organic sources

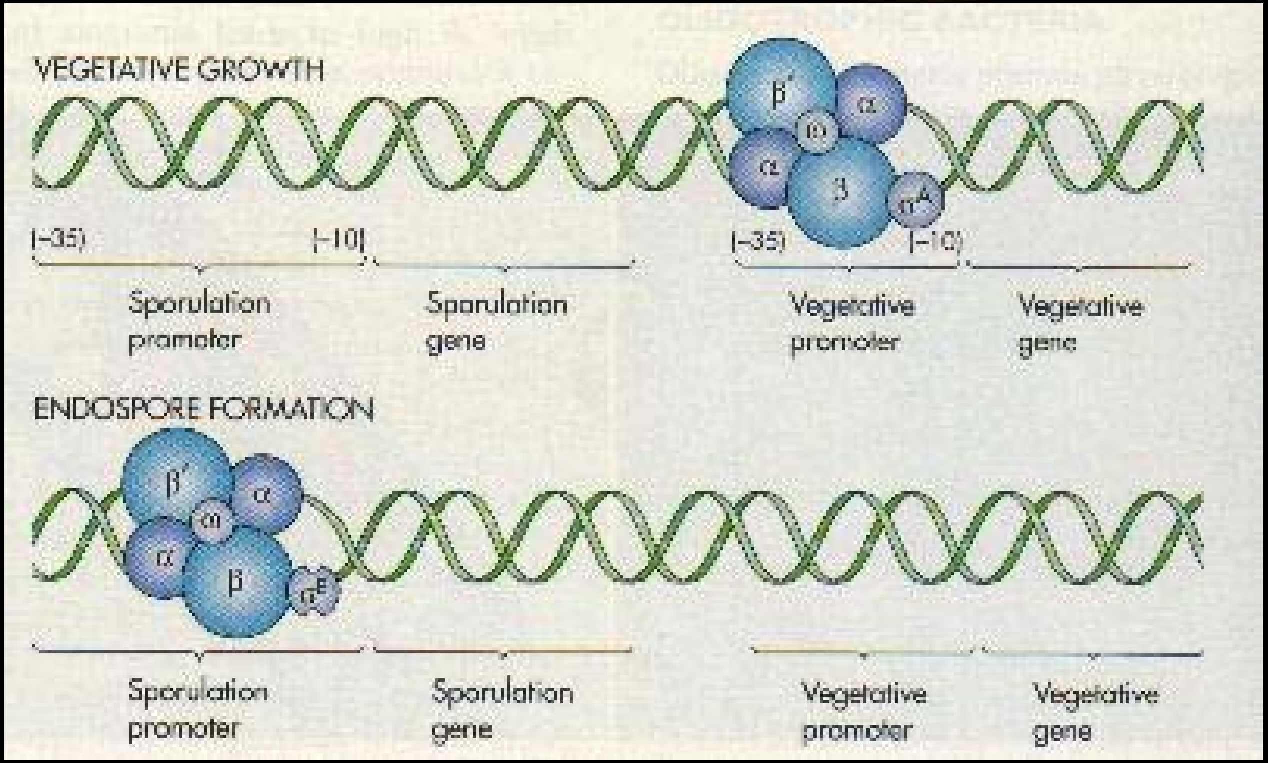
Specific Strategies for Survival in Conditions of Low Nutrients

- Oligotrophs
  - bacteria that preferentially grow at low nutrient levels
  - generally have slow growth rates
  - can acquire substrates against steep concentration gradients
  - conserve available resources
  - generally small cells
  - produce prosthecae which increases the surface area to volume ratio

Endospore-Forming Bacteria

- Differentiation to a non-reproducing form
- Endospore formation is repressed by glucose and other growth substances
- Energy for sporulation comes from cellular protein and poly-β-hydroxybutyrate
- Spore-forming organisms have specific genes responsible for spore formation
  - Expression is controlled by sigma factors

Genetics of Endospore Formation



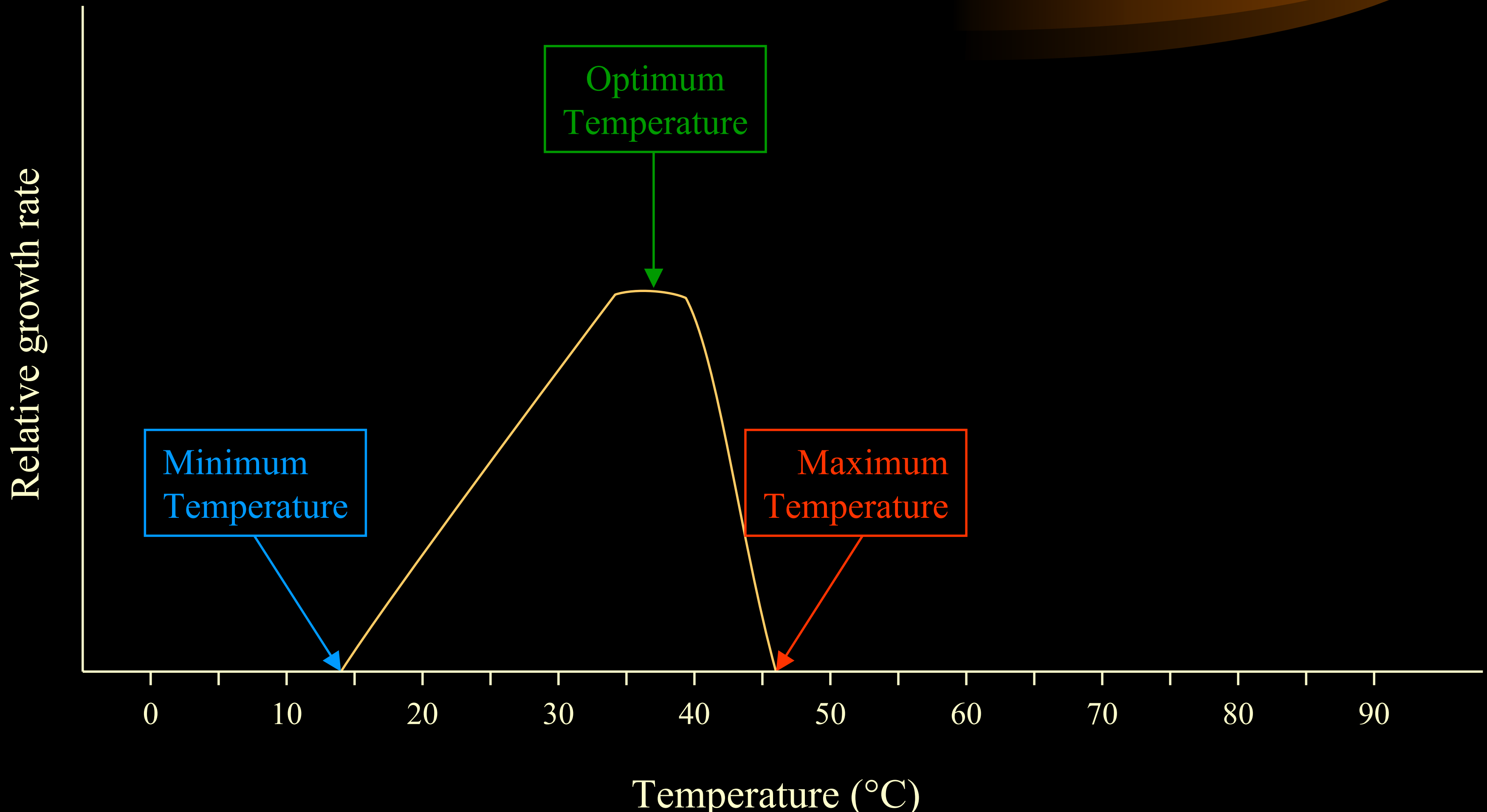
Endospore properties

- Can withstand adverse conditions of dessication and elevated temperatures
  - Can remain viable almost indefinitely
- Under permissive conditions of water and nutrient availability and acceptable temperature, spores germinate
  - Spore swells
  - Breaks out of spore coat
  - Elongates
  - Returns to a vegetative cell

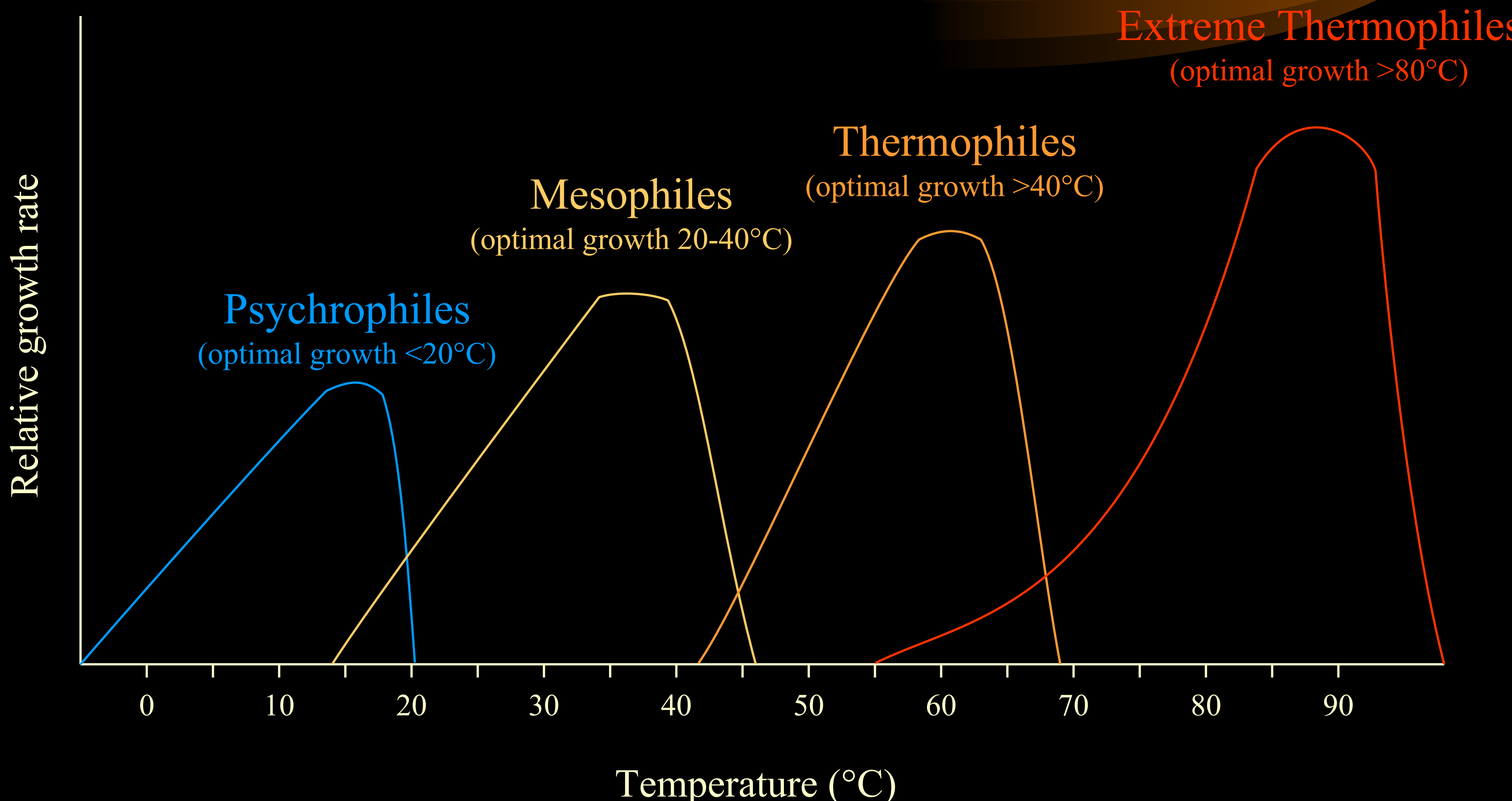
Effects of Temperature

- One of the most important factors influencing bacterial growth
- Specific cells growth within well-difined temperature growth ranges
  - Difined by…
    - Minimum temp.
      - Metabolic inactivity below this temp
    - Maximum temp
      - Cells don’t grow above this temperature
    - Optimal temperature
      - Highest growth rate

Growth Ranges



Growth Ranges



Psychrophiles

- Optimal growth below 20°C
- Can grow below 0°C (if liquid water is available)
- Found in Artic, Antarctic and ocean environments
- Can be found in refrigerators
  - Cause food spoilage

Psychrophile Physiology

- Enzymes and ribosomes are active at low temps
  - Can be inactivated at moderate temps (~25°C)
- More lipids with unsaturated or short chain fatty acids in psychrophile membranes
  - Membranes more semifluid in the cold
  - Under higher temps, membranes become leaky
- Limit of psychrophile growth and metabolism may be the availability of liquid water

Mesophiles

- Optimal growth between 20-40°C
  - Many have optimal temps of 37°C
    - Physiological temperature for humans
- Normal body flora and most pathogens are mesophiles
  - Grow rapidly and optimally in the human body
  - Eg. *E. coli*

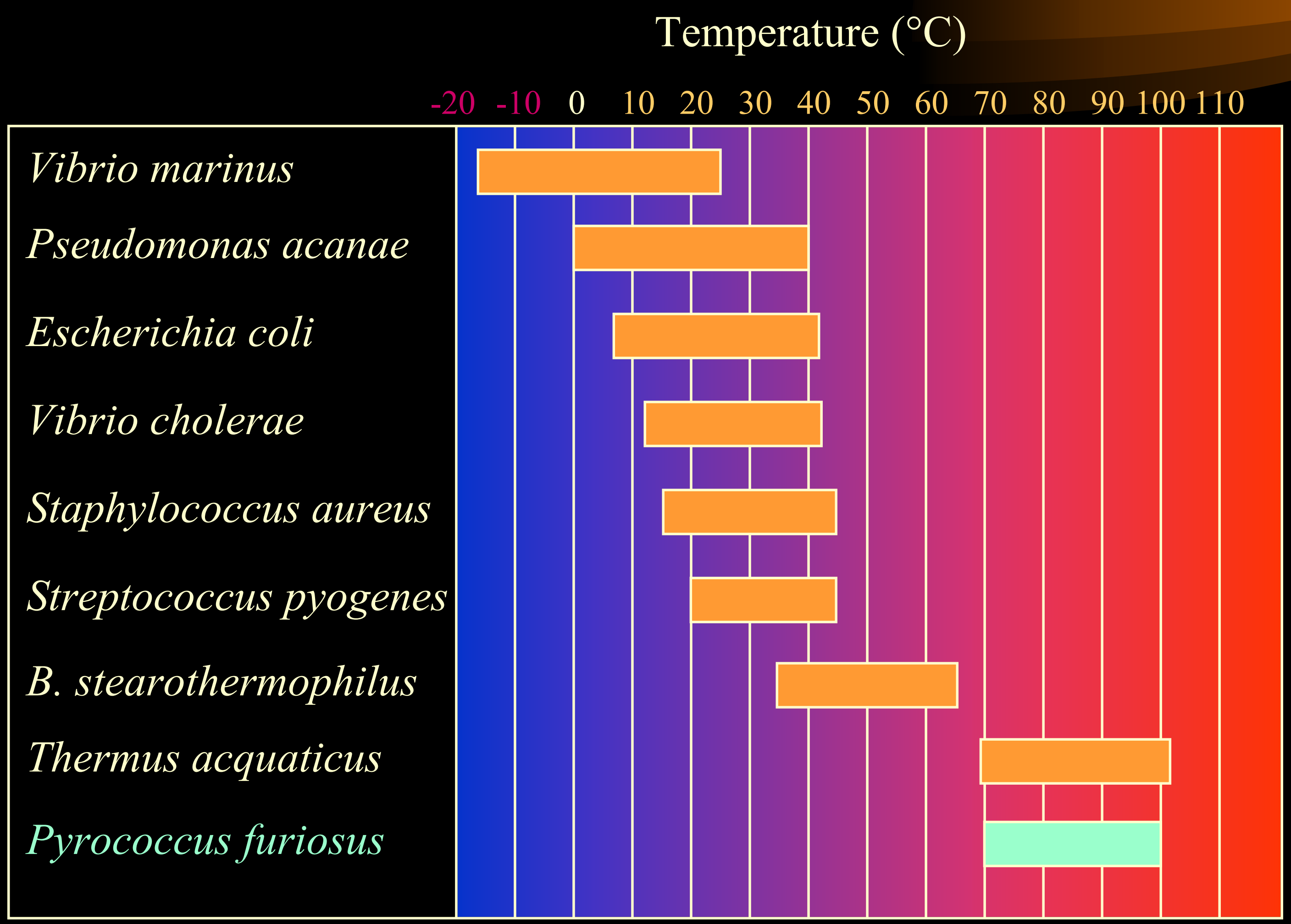
Thermophiles and Extreme Thermophiles

- Optimal growth for thermophiles: >40°C
  - many have optima between 55-60°C
- Optimal growth for extreme thermophiles: > 80°C
- Sources
  - hot springs
  - hydrothermal vents
- Many thermophiles can survive low temperatures
  - viable thermophilic bacteria found in frozen Antarctic soil

Physiology of Thermophiles

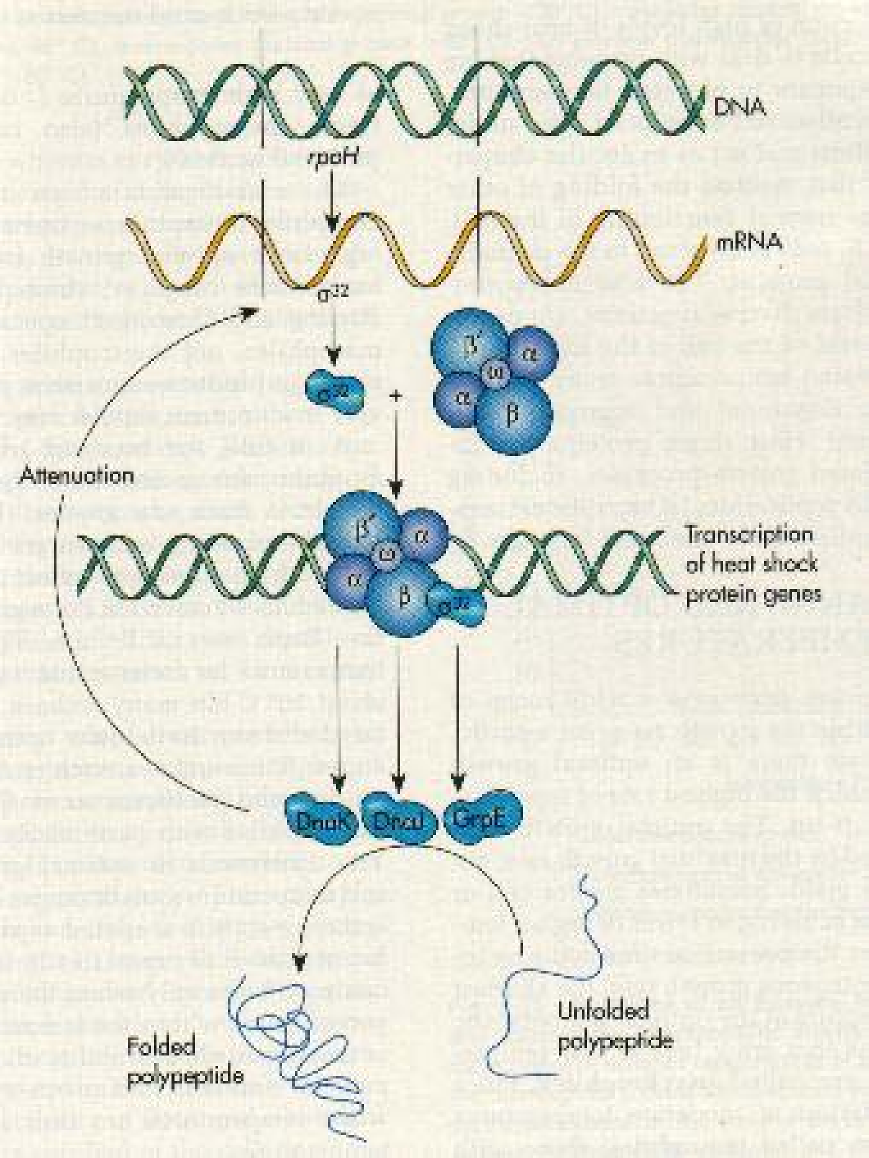
- Enzymes are not readily denatured at high temps
  - possibly due to specific primary sequences
- Membranes posses a major proportion of high molecular weight and branched fatty acids
  - membranes remain semipermeable at high temps

Temperature Growth Ranges: Examples



Heat Shock Response

- Occurs when organisms are shifted to a higher temperature
  - evolutionarily conserved response
- Results in the production of a set of heat shock proteins (Hsps)
  - 24 proteins in E. coli
  - transcription of 20 of these is under the control of $\sigma 32$
    - encoded by rpoH


DNA
rpoH
mRNA
σ^{32}
Attenuation
Transcription of heat shock protein genes
DnaK
DnaJ
GrpE
Folded polypeptide
Unfolded polypeptide

Roles of HSPS

- Protect proteins against degradation at elevated temperatures
  - denatured proteins aggregate and become non-functional
- Involved in all growth-related processes
  - cell division
  - replication
  - transcription and translation
  - protein folding
  - membrane function

Lethal Effects of Temperature

- Lethal effects of heat
  - Heat sterilisation
    - 121°C for 15 minutes for steam sterilisation
    - 180°C for 180 minutes for dry heat sterilisation
- Lethal effects of cold
  - if >0°C, but less than minimum growth, bacteria will lose viability due to absence of growth
  - formation of ice crystals can destroy cells
    - freeze fracturing

Effects of Oxygen

- Classification of microorganisms based on O_2 tolerance or requirement
- Aerobes
  - Obligate aerobes
    - absolute requirement for molecular O_2
    - carry out aerobic respiration
  - Microaerophiles
    - grow only in low concentrations of O_2
    - O_2 is an absolute requirement

Anaerobes

- Anaerobes
  - Obligate anaerobes
    - O_2 is inhibitory to microbial growth
    - carry out fermentation
  - Strict anaerobes
    - very sensitive to O_2
    - die even with a short exposure
  - Facultative anaerobes
    - grow in the presence or absence of O_2
    - $+O_2$: aerobic respiration
    - $-O_2$: fermentation

Oxygen Toxicity

- Relationship between organisms and O_2 can be more than metabolic
- other factors include the formation of toxic O_2 products and the availability (or absence) of enzymes to deal with these products
  - catalase and peroxidase degrade peroxides
  - superoxide dismutase degrades superoxides
- Reduced O_2 may arise from reduced flavoproteins (and other electron acceptors) and as a result of radiation

Anaerobiosis

- Ability of some facultative anaerobes (such as *E. coli*) to carry out aerobic respiration using oxygen at the terminal electron acceptor when molecular oxygen is available
- Can also use anaerobic respiration using nitrate (or other terminal electron acceptors) when oxygen levels are depleted
- Regulated by the Arc and Fnr systems

Osmotic Pressure and Salinity

- Osmotic pressure
  - diffusion of water across cell membranes in response to solute concentrations
  - often associated with saline environments
- Hypertonic solutions can lead to cell shrinkage and dessication
- Hypotonic solutions can result in cell bursting
- Osmotolerant
  - organisms that can withstand high osmotic pressures
- Osmophiles
  - require high solute concentrations for growth

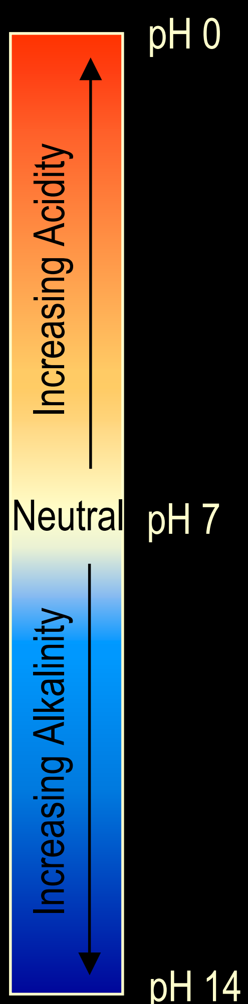
Halophiles

- Salinity has important effect on osmotic pressure
- Halophiles
  - require NaCl
  - moderate halophiles (3% NaCl)
  - extreme halophiles (upto 25% NaCl)
- High NaCl concentrations normally disrupt membrane transport systems and denature proteins
  - some organisms, such as *Halobacterium*, possess unusual plasma membranes and many unusual enzymes

Hydrostatic Pressure

- The pressure exerted by a column of water as a result of the wieght
- Each 10m water depth = 1 atm.
- Hydrostatic pressures > 200 atm generally inactivate enzymes and disrupt membrane processes
- barotolerant
  - can grow at high hydrostatic pressures
- barophiles
  - grow best at high pressures

Effects of pH



- Bacteria exhibit various tolerances to pH
- pH effects are largely based on changes in the nature of proteins
  - Effects charge interactions within and between polypeptides
    - Effect secondary and tertiary structure of proteins
- Most grow in a range between 6.0 and 9.0
  - Neutralophiles
    - Grow best under neutral conditions

Acidophiles

- Restricted to growth at low pH
- Acidophilic membranes cannot function under neutral conditions
- Physiology
  - Internal pH of all cells is relatively neutral
  - If pH of environment is less than the pH of the cytoplasm (i.e. a large ΔpH), it will be hard to generate the PMF required for ATP synthesis
  - Many acidophiles have a reverse membrane potential ($\Delta\Psi$, charge separation across the membrane)
    - Outside of membrane is negatively charged
    - Inside of membrane is positively charged
  - Combination of large ΔpH and reversed $\Delta\Psi$ generate ATP via PMF

Alkaliphiles

- Bacteria with high pH requirements for growth
  - Can be in the range of 9 to 11
- Physiology
  - Generation of PMF
    - Reversed ΔpH across the membrane
      - 7 to 9 internally, 9 to 11 extrenally
    - Alkaliphiles use Na^+/H^+ or K^+/H^+ antiporters to maintain ΔΨ sufficiently high to drive the PMF

pH Tolerances of Various Bacteria

Organism	Minimum pH	Optimum pH	Maximum pH
Thiobacillus thiooxidans	1.0	2.0-2.8	4.0-6.0
Lactobacillus acidophilus	4.0-4.6	5.8-6.6	6.8
Escherichia coli	4.4	6.0-7.0	9.0
Clostridium sporogenes	5.0-5.8	6.0-7.6	8.0
Nitrobacter spp.	6.6	6.6-8.6	10.0
Nitrosomonas spp.	7.0-7.6	8.0-8.8	9.4

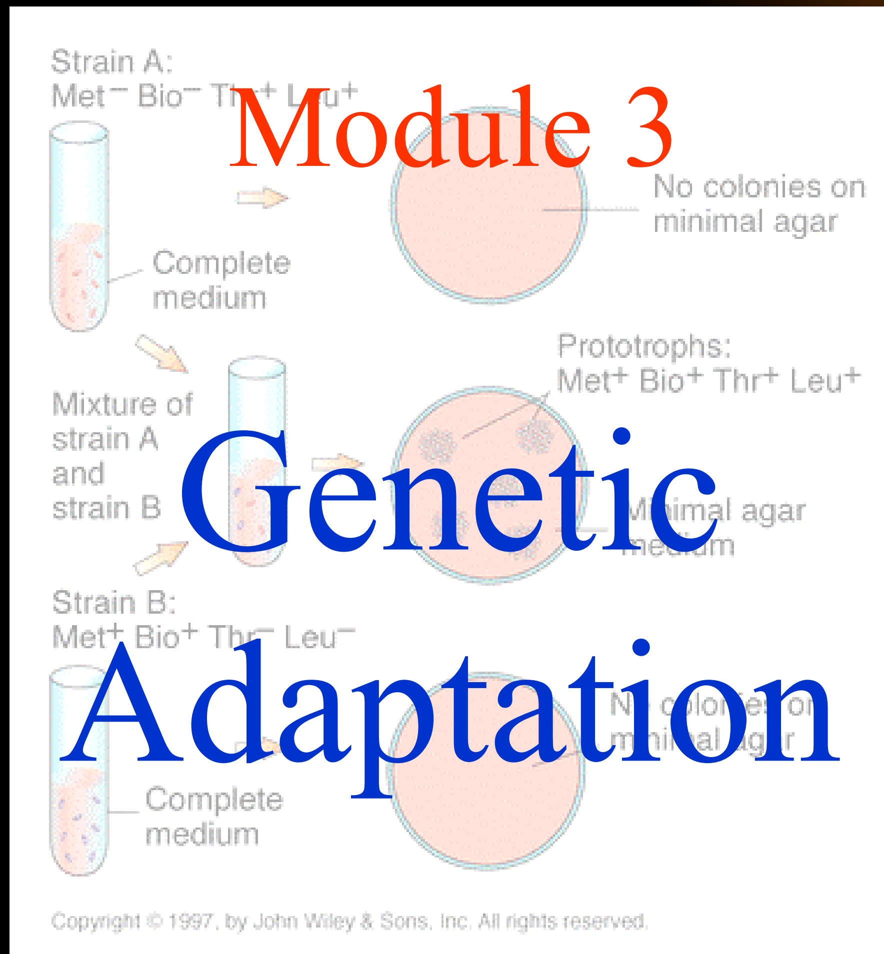
Effects of Light

- Light is required by photosynthetic bacteria to generate ATP
- Function optimally at specific light intensities and utilise specific wavelengths
- Some can move through their environment in response to light (phototaxis)
- Light (in particular UV light) can have detrimental effects
  - Some organisms synthesise carotenoids and pigments that absorb harmful wavelengths before they can cause damage

Learning Exercises

- Read *Principles of Microbiology* (Atlas) Chapter 9
- Find examples for each group discussed in this module
  - Eg. Alkaphiles, barophile, barotolerant etc

Next Week



Module 3

Genetic Adaptation