Slide 1

# Microbial Growth

Lecture 9

Slide 2

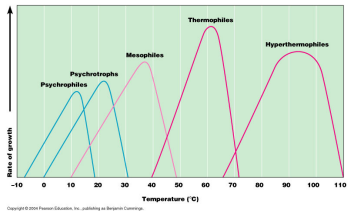
## Learning Outcomes

- What a Microbe Wants
  - Requirements for Growth
- Growing microbes in Lab conditions
- Oxygen requirements
- Bacterial Growth
- Counting Colonies

Slide 3

# Temperature and Tolerance

- Minimum
  - Lowest temp of growth
- Optimum
  - Fastest rate of growth
- Maximum
  - Highest temp of growth



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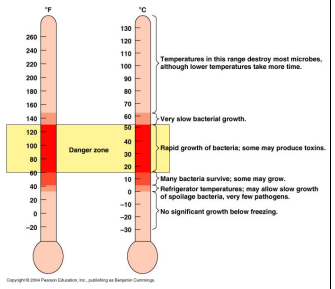
Slide 4

# Cold Tolerant Bacteria

- Psychrophiles
- Can grow at 0 °C,
  optimum = 15 °C
  max <25 °C
- Psychrotrophs
- Can grow @ 0 °C
  - optimum 20-30 °C
  - Max 40 °C
  - *Listeria monocytogenes*
- Refrigeration
  - Stops mesophile growth
  - Slows psychrotrophs



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## Meet the Mesophiles

- Optimum 25-35 °C
- Most common Pathogens
- Optimum range = host body temp
- Responsible for:
  - most food spoilage
  - Most diseases

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## Meet the Thermophiles

- Optimum 50-60 °C
- Minimum for some ~45 °C
- Endospores very heat resistant
  - Compost
- Volcanic Hot Springs
  - *Thermus aquaticus*,
- Extreme thermophiles - Archaea
  - Optimum 80 °C
- Hydrothermal vents

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## Acidophiles

- Most microbes prefer neutral pH
- 6.5-7.5
- Very few pH <4
- Lactobacilli ferments sugars → Lactic Acid
- Lowers pH
  - *Lactobacillus acidophilus*
  - Vaginal flora
- Pickling preserves vegetables
- Yogurt and cheese

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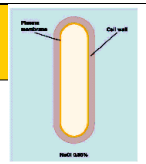
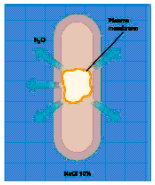
## Osmotic Pressure

- Microbes
- 80-90% water
- Growth Media
- ~1.5%
- Hypertonic solution
  - Water drawn out by osmosis
- → plasmolysis
- Preservation
  - Salting
  - Jam (adding sugar)
  - Drying
- Halophiles
- Salt tolerant
  - *Staphylococcus epidermidis*
- Extreme Halophiles
  - *Halobacterium*
- Grow in salt ponds



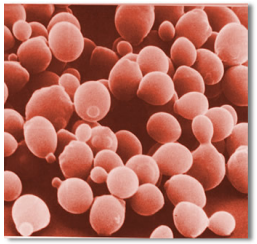
## Sources of Carbon

- Carbon = 50% dry weight of bacteria
- Organic carbon
  - Chemoheterotrophs
- Inorganic = $CO_2$
  - Chemoautotrophs
  - Photoautotrophs



## Oxygen

- Obligate Aerobes
- Oxygen [conc] in water low
- Facultative Anaerobes
  - Can use anaerobic respiration when [$O_2$] low
    - Coliforms (*E. coli*)
    - Yeast



## Oxygen Toxicity

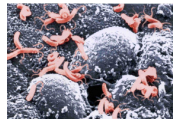
- Obligate Anaerobes
- $O_2$ toxic:
  - *Clostridium*
- Singlet Oxygen $O_2$
  - Phagocytic cells
- Superoxide free radicals $O_2^-$
  - SOD Superoxide Demutase
  - $O_2^- + O_2 + 2H^+ \rightarrow H_2O_2 + O_2$
- Peroxide anion $O_2^{2-}$
  - Catalase
  - $2H_2O_2 \rightarrow 2\ H_2O + O_2$

http://www.astaxanthin.org/oxidation.htm



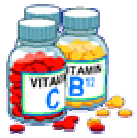
## Oxygen Tolerance

- Aerotolerant Anaerobes
  - Cannot use $O_2$ but tolerant
  - Can be grown without special treatment required for obligate anaerobes
  - Possess SOD
    - *Lactobacilli*
    - *Streptococcus*
    - *Enterococcus*
- Microaerophiles
  - Aerobes the prefer low $[O_2]$
  - Deep stab in solid medium
  - *Helicobacter*



## Organic Growth Factors

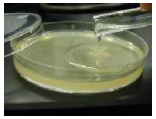
- Essential organic compounds
- Microbes cannot synthesize
- Vitamins
  - → Coenzymes
- Amino acids
- Pyramidines
  - Thymine + Cytosine
- Purines
  - Adenine + Guanine






## Culture Media Terminology

- Culture medium – nutrient material prepared to grow microbes
  - Undefined
  - Defined
- Inoculum – introduced microbes
- Culture – growth of microbes after incubation peroid
- Sterility – no other microbes in media
- Agar – solidifying agent



# Growth Media

TABLE 6.5 **Culture Media**

| Type | Purpose |
|---|---|
| Chemically defined | Growth of chemoautotrophs and photoautotrophs, and microbiological assays. |
| Complex | Growth of most chemoheterotrophic organisms. |
| Reducing | Growth of obligate anaerobes. |
| Selective | Suppression of unwanted microbes; encouraging desired microbes. |
| Differential | Differentiation of colonies of desired microbes from others. |
| Enrichment | Similar to selective media but designed to increase numbers of desired microbes to detectable levels. |

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# Undefined Media

- Recipe 1
- 2 slices white bread
- 2 slices meat
- 1 squirt mayo
- 2 pieces of lettuce
- 2 slices tomato

- Recipe 2
- 2 slices white bread
- 2 slices honey smoked ham
- 1 squirt mayo
- 2 pieces of lettuce
- 2 slices tomato

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## Selective Media

- May be liquid or solid
- Contains a chemical additive that inhibits the growth of one specific group of microbes
- Maconkey
  - inhibits Gram +
- PEA
  - Inhibits Gram –

Figure 4-4A

Figure 4-4B

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## Differential Media

- Contains an indicator to detect a particular metabolic process
- Fermentation
  - Mannitol Salt Agar
  - *Staphylococcus aureus* ferments Mannitol
  - Acid byproducts lower pH
  - pH indicator phenol red changes color at 6.8

Figure 4-2

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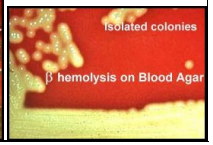
## Blood Agar

- Culturing bacteria with hemolytic enzymes
- Alpha hemolysis
  - *Streptococcus pneumoniae*
- Beta hemolysis
  - *Strep pyogenes*



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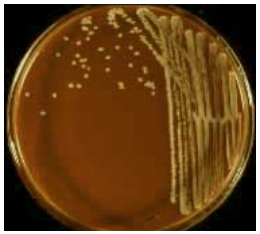
## Chocolate Agar

- Chocolate Agar
  - Factor X (hemin)
  - Factor V (NAD+)
- Fastidious Organisms
- Require additional nutrients for growth
- *Neisseria,*
- *Haemophilus*
- *Legionella*



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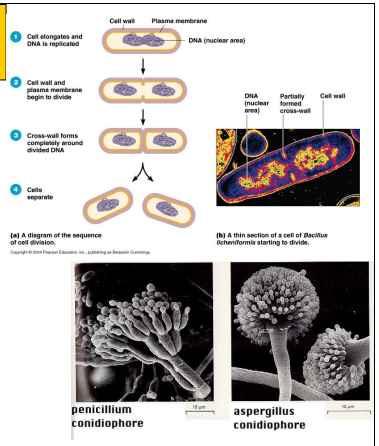
## Bacterial Growth

- Multiplication by Division
- Binary fission
  - Most bacteria
- Conidiospores
  - Actinomycetes
  - Also Molds






penicillium conidiophore



aspergillus conidiophore

# Generation Time

- Time for a cell to divide
- Most bacteria 1-3 hours
- E.coli –
  - 20min ideal conditions
  - 24 hours in human

| Numbers of Cells | Numbers Expressed as a Power of 2 | Visual Representation of Numbers |
|---|---|---|
| 1 | $2^0$ | • |
| 2 | $2^1$ | •• |
| 4 | $2^2$ | •••• |
| 8 | $2^3$ | •••••••• |
| 16 | $2^4$ | •••••••••••••••• |
| 32 | $2^5$ | •••••••••••••••••••••••••••••••• |

(a)

| Generation Number | Number of Cells | $Log_{10}$ of Number of Cells |
|---|---|---|
| 0 | 1 | 0 |
| 5 ($2^5$) = | 32 | 1.51 |
| 10 ($2^{10}$) = | 1,024 | 3.01 |
| 15 ($2^{15}$) = | 32,768 | 4.52 |
| 16 ($2^{16}$) = | 65,536 | 4.82 |
| 17 ($2^{17}$) = | 131,072 | 5.12 |
| 18 ($2^{18}$) = | 262,144 | 5.42 |
| 19 ($2^{19}$) = | 524,288 | 5.72 |
| 20 ($2^{20}$) = | 1,048,576 | 6.02 |

(b)

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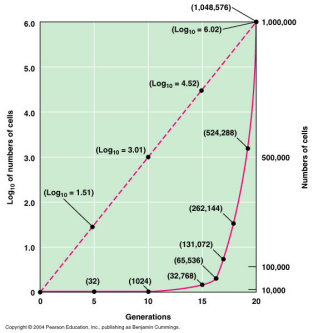
# Logarithmic Representation

- Advantages
  - Small and large numbers of cells can be graphed
- Disadvantages
  - Logarithms = math

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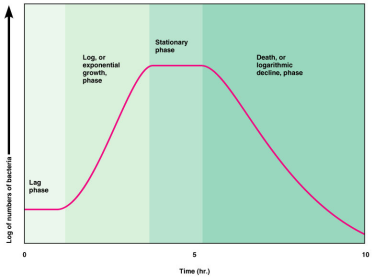
# Phases of Growth

- Lag Phase
  - Cells do not immediately multiply hours-days
- Log Phase
  - Exponential growth
- Stationary Phase
  - Growth rate slows deaths = new cells
- Death Phase
  - Deaths > new cells
- Continuous Culture
- Renew resources to extend log phase
- Commercial production

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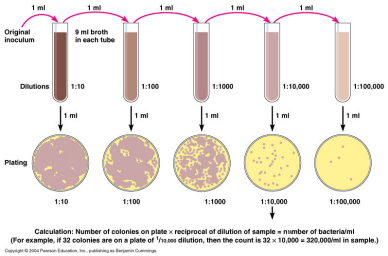
# Direct Measurement of Microbial Growth

- Serial Dilutions
- Spread Plates
- Plate Counts

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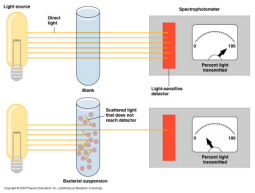
http://www.accessexcellence.org/AE/AEPC/WWC/1993/serial.html

- Turbidity
  - Greater number of cells = greater light scattering
- Metabolic activity
  - Acid or $CO_2$ production
  - Proportional to cell number
- Dry weight
  - Filamentous bacteria and molds

## Indirect Estimates