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اهداف اختصاصی: فیزیولوژی میکروارگانیسم‌ها

پس از پایان این جلسه انتظار می‌رود فراگیر:

- اهمیت مراحل مختلف رشد در بیماری‌زاپی ابزاری را با بیان کند.

- قادر به افتراق باکتری‌های هوازی اجباری، بی‌هوازی اختیاری و بی‌هوازی اجباری از هم باشد.

- علت مطالعه مشابه در میکروب شناسی پزشکی را بیان نماید.

- تنفس هوازی، بی‌هوازی و تخمیر را از هم افتراق داده و کارایی آنها در تولید انرژی بیان نماید.

- نقش واکنش های تخمیری در ایجاد علائم بیماری و تشخیص میکروارگانیسم‌ها را توضیح دهد.

- عملکرد پلاسمید‌ها و ترانسپوزون‌ها را توضیح دهد.

- مکانیسم‌های انتقال ژن در باکتری‌ها را با ذکر مثال با هم مقایسه نماید.

- اهمیت بالینی تبادلات زنندری در بین باکتری‌ها را با ذکر مثال توضیح دهد.

- جهش را تعیین نموده و انواع آن را بیان کند.

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Cultivation of Microorganisms

- Biology of the organism
- The site of the infection
- The patient’s immune response to the infection
- The quality of the culture media
Any substance, that must be provided to an organism

1- Macronutrients

- Required in large quantities
- Cell structure & metabolism
- C, O, H, N, P, S
- Mg$^{2+}$, Ca$^{2+}$, Fe$^{2+}$, K$^+$, Na$^+$, Cl$^-$

2- Micronutrients

- Trace elements
- Much smaller amounts
- Enzyme function & Protein structure
- Co, Mn, Ni, Zn, Mo, Se
Major nutritional types

All Organisms

Chemical Energy

- Chemotrophs
  - Electron Source: Inorganic
  - Carbon and Electron Source: Organic
    - Lithotrophs
    - Organotrophs
      - Carbon Source: CO₂

Light Energy

- Phototrophs
  - Carbon Source: CO₂

- Autotrophs
Growth factors

✓ Organic compounds

✓ Essential cell components or their precursors

✓ Cannot be synthesized by the organism

(1) Amino acids

(2) Purines & pyrimidines

(3) Vitamins

*Haemophilus influenzae*

Hemin (factor X), NAD (factor V), thiamine
Prototroph

Growing on a minimal medium

Consisting a simple carbohydrate or CO₂ carbon source
with inorganic sources of all other nutrient requirements

Auxotroph

Requiring one or more complex organic nutrients

Amino acids, nucleotide bases, enzymatic cofactors
1. Enriched nonselective media

- Support the growth of most non fastidious organisms
  - Blood agar
  - Chocolate agar
  - Mueller-Hinton agar: Bacterial susceptibility test medium
  - Thioglycolate broth: for anaerobic bacteria

2. Selective media & differential media

- MacConkey agar
  - Selective for gram-negative bacteria
  - Differential for lactose-fermenting species

- Mannitol salt agar
  - Selective for staphylococci
  - Differential for *Staphylococcus aureus*

- Lowenstein-Jensen (LJ) medium
  - Selective for mycobacteria
3. Specialized media

- Buffered charcoal yeast extract (BCYE) agar
  - Recovery of *Legionella* and *Nocardia*

- Lim broth
  - Recovery of *Streptococcus agalactiae*

- Thiosulfate citrate bile salts sucrose (TCBS) agar
  - Recovery of *Vibrio* species
Growth takes place on two levels

1- Increase in cell size

2- Increase in cell number

- Results when cells simply become longer or larger
- Population growth rather than growth of individual cells
Growth of Bacterial Populations

- **Liquid medium**

- **Batch culture or closed system**
  - No fresh medium is provided
  - Nutrient concentrations decline
  - Concentrations of wastes increase

The Growth Curve

- **Lag phase**
- **Exponential growth phase**
- **Stationary phase**
- **Death phase**

Total cells in population, live and dead, at each phase.
$M = M_i \cdot 2^n$

$M =$ number of bacteria at the end of the time interval

$M_i =$ number of bacteria at the beginning of a time interval

$n =$ number of generations (number of times the cell population doubles during the time interval)
Stationary Phase

- Total number of viable bacteria does not change
- Changes in gene regulation
- Quorum sensing induced
- Biofilm formation
- Up regulation of virulence factors
- Spore formation
- Cell differentiation

Species specific
Environmental Factors

- Water activity \( (a_w) \)
- Osmotic Pressure
- Temperature
- O\(_2\)
- pH
- Others: atmospheric pressure, radiation
- **Psychrophiles**
- **Mesophiles**
- **Thermophiles**

**Psychrotolerant**
Grow at 0°C (optima of 20°C to 40°C)
## Oxygen ($O_2$)

<table>
<thead>
<tr>
<th></th>
<th>Obligate aerobes</th>
<th>Facultative anaerobes</th>
<th>Aero tolerant anaerobes</th>
<th>Obligate anaerobes</th>
<th>Microaerophiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>oxygen (~20%)</td>
<td>prefer oxygen</td>
<td>ignore oxygen</td>
<td>oxygen is toxic</td>
<td>&lt; 2 - 10% oxygen</td>
</tr>
<tr>
<td></td>
<td>+ SOD + Catalase</td>
<td>+ SOD + Catalase</td>
<td>+ SOD - Catalase</td>
<td>- SOD - Catalase</td>
<td>+ SOD +/- Catalase (low levels)</td>
</tr>
</tbody>
</table>

- **SOD** stands for Superoxide Dismutase
- **Catalase** is an enzyme that breaks down hydrogen peroxide into water and oxygen.
Toxic Forms of Oxygen

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- \quad \text{Superoxide} \\
O_2^- + e^- + 2 \text{H}^+ & \rightarrow \text{H}_2\text{O}_2 \quad \text{Hydrogen peroxide} \\
\text{H}_2\text{O}_2 + e^- + \text{H}^+ & \rightarrow \text{H}_2\text{O} + \text{OH}^- \quad \text{Hydroxyl radical} \\
\text{OH}^- + e^- + \text{H}^+ & \rightarrow \text{H}_2\text{O} \quad \text{Water}
\end{align*}
\]

**Overall:** \[O_2 + 4 \text{e}^- + 4 \text{H}^+ \rightarrow 2 \text{H}_2\text{O}\]

(a) Catalase:
\[\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2\]

(b) Peroxidase:
\[\text{H}_2\text{O}_2 + \text{NADH} + \text{H}^+ \rightarrow 2 \text{H}_2\text{O} + \text{NAD}^+\]

(c) Superoxide dismutase:
\[O_2^- + O_2^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + O_2\]

(d) Superoxide dismutase/catalase in combination:
\[4 O_2^- + 4 \text{H}^+ \rightarrow 2 \text{H}_2\text{O} + 3 O_2\]

(e) Superoxide reductase:
\[O_2^- + 2 \text{H}^+ + \text{cyt c}_{\text{reduced}} \rightarrow \text{H}_2\text{O}_2 + \text{cyt c}_{\text{oxidized}}\]
Anaerobic Culture Methods

- Reducing media
  - Thioglycollate or oxyrase that combine $O_2$
  - Heated to drive off $O_2$
- Anaerobic jar
Capnophiles

Candle Jar

CO$_2$-packet

Petri plate with bacterial culture

Gas generator
Why Metabolism is important?

- Identification
- Microbial application
- Chemotherapies
Metabolism

**Metaballein**

*Change*

The breakdown of complex organic materials releases energy (Exergonic).

Breakdown of complex organic
Releases ENERGY

The building of complex organic requires energy (Endergonic).

The building of complex organic
Requires ENERGY

**Amphibolic**
Metabolic pathway

Is a series of chemical reactions occurring within a cell

Primary metabolic pathway

Reactions that do the basic work of the cell
Get food & grow

Primary metabolites

Is directly involved in normal growth, development, & reproduction

Secondary metabolites

Are organic compounds
Are not directly involved in the normal growth, development, or reproduction
**Metabolism & Energy**

Cells Can't Eat Hamburgers

![Adenosine monophosphate (AMP), Adenosine diphosphate (ADP), Adenosine triphosphate (ATP)]

- Bond that releases energy when broken
Metabolic Pathways of Energy Production

Organic e⁻ donor

- Fermentation
  - Endogenous organic electron acceptor

- Aerobic respiration
  - O₂

- Anaerobic respiration
  - NO₃⁻, SO₄²⁻, CO₂, fumarate

Chemical work
  - Transport work
  - Mechanical work

Respiration
  - Fermentation
  - Photosynthesis
  - Chemoautotrophy

ATP

ADP + Pᵢ
Carbohydrate Catabolism

Glycolysis
  Glucose
  Pyruvic acid

Fermentation
  Respiration
Most common pathways of glucose metabolism

**AEROBIC RESPIRATION**

- Glycolysis
  - Glucose (6 C)
  - ATP
  - NADH
  - 2 pyruvate (3 C)
  - CO₂
  - Acetyl CoA
  - Krebs cycle
  - FADH₂
  - NADH
  - ATP
  - Electrons
  - Electron transport

- O₂ is final electron acceptor.
- ATP produced = 38

**ANAEROBIC RESPIRATION**

- Glycolysis
  - Glucose (6 C)
  - ATP
  - NADH
  - 2 pyruvate (3 C)
  - CO₂
  - Acetyl CoA
  - Krebs cycle
  - FADH₂
  - NADH
  - ATP
  - Electrons
  - Electron transport

- Nonoxygen electron acceptors (examples: SO₄²⁻, NO₃⁻, CO₃²⁻)
- ATP produced = 2 to 36

**FERMENTATION**

- Glycolysis
  - Glucose (6 C)
  - ATP
  - NADH
  - 2 pyruvate (3 C)
  - CO₂
  - Acetyl CoA
  - Krebs cycle
  - FADH₂
  - NADH
  - ATP
  - Electrons
  - Electron transport

- Fermentation
  - Lactic acid
  - Acetaldehyde
  - Ethanol
  - Or other alcohols, acids, gases

- ATP produced = 2

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Anaerobic Respiration

- Final electron acceptor is an *inorganic substance* other than O₂
- Only part of the Krebs cycle operates
- Not all the carriers in the ETC participate in anaerobic respiration

Nitrite ion (NO₂⁻), nitrous oxide (N₂O), or nitrogen gas (N₂)

- *Pseudomonas & Bacillus*

  Sulfate (SO₄²⁻): is reduced to
  - Hydrogen sulfide (H₂S)

- *Desulfovibrio*

  Carbonate (CO₃²⁻)
  - Methane (CH₄)

<table>
<thead>
<tr>
<th>Electron Acceptor</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td>NO₂⁻, N₂ + H₂O</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>H₂S + H₂O</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>CH₄ + H₂O</td>
</tr>
</tbody>
</table>
NADH is oxidized to NAD

The electron acceptor is pyruvate or a pyruvate derivative

The substrate is partially oxidized

ATP is formed by substrate-level phosphorylation
Fermentation

1. Food & industrial applications
2. Diagnostic applications
3. Disease
Fermentation products

1- Mixed acid fermentation

- Acetic, Lactic, Succinic, & Formic acids
- Methyl red test (MR)
- *Escherichia, Shigella, Salmonella, Proteus*

2- Butanediol fermentation

- 2,3- butanediol
- Voges-Proskauer (VP)
- *Enterobacter, Serratia, Erwinia*
Carbohydrate Catabolism

Glycolysis (EMP)

- Glucose
  - Glucose 6-P
    - Fructose 6-P
      - Fructose 1,6-P
        - 2 Glycer aldehyde 3-P
          - Net yield: 2 Pyruvate
            - 2 ATP
              - 2 NADH

Entner-Doudoroff (ED)

- Sugar acids
  - 6-P-gluconate
    - 2-Oxo-3-deoxy-6-P-gluconate
      - Ribulose 5-P
        - CO₂
          - Sugar phosphates: 7C 6C 3C 4C
            - Biosynthesis
              - ATP
              - NADH, NADPH

Pentose-Phosphate Shunt (PPS)

- ATP
- NADH, NADPH
- 2NADPH

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**Bacterial Genome**

**Bacterial Chromosome**

**Bacterial Extrachromosomal DNA**

- Marked downsizing in isolated intracellular niches
- Accumulation of pseudogenes and insertion elements after shift to new niche
- Gene duplication
- Horizontal gene transfer by phages, plasmids and pathogenicity islands
- Rapid emergence of genetically uniform pathogens from variable ancestral populations
- Recombination and rearrangement
- Patho-adaptation
- Single-nucleotide polymorphisms

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Bacterial chromosomal DNA

- Circular, double-stranded DNA
  - Some species: have more than one
  - Some species: linear
  - Some species: linear & circular

- Size
  - \( \sim 5 \times 10^6 \) base pairs / genome

Necessary genes

- Bacterial growth & reproduction
  - Operons
  - Islands (pathogenicity islands)
A functioning unit

Promoter  Operator  Structural genes

Transcription of polycistronic RNA
A collection of operons

Regulation by the same regulatory protein
1- Plasmids

2- Bacteriophage

3- Mobile genetic elements

- Insertion sequences: IS

- Transposons: Tn
Plasmids

- Double stranded DNA, are usually circular
  - Linear in *Borellia*
- Replicons: are autonomously replicating
- 1500 to 400,000 base pairs
- Can be transferred between cells
- Episomes: can integrate into the host chromosome
1- Fertility plasmids (F-factor)
   F+/ F-; Hfr (high frequency of recombination)

2- Resistance plasmids (R-factor)
   Antibiotic resistance

3- Virulence plasmids
   Adhesion, toxin

4- Col-plasmids
   Colicins gene → bacteriocins

5- Metabolic plasmids
   Enzymes, sugar (lactose)

6- Degradative plasmids
   Digestion of unusual substances, e.g., toluene or salicylic acid
Viruses that infect bacteria

Lysogenic growth

“Temperate“ phage
The phage genome: progeny
Bacterial cell called: lysogen

Lytic growth

“Virulent “ phage
Packaging of phages
Production of lysis enzymes
Phages release from bacterial cells
**Mobile genetic elements**

- **Insertion sequences (IS)**
- **Transposons**
- Move from one site in DNA to another (transposition)
- Part of other genetic elements (chromosome, plasmid)

![Diagram showing replicative and conservative transposition](image-url)
Transposons (Jumping genes)

- Transposase + 2 inverted repeats of DNA sequences

Gene coding for the enzyme required for transposition (transposase)

Inverted repeat

Insertion sequence (IS)

Antibiotic-resistance gene
Importance

✓ Transfer of antibiotic resistance gene
✓ Transfer of virulence gene

Mechanisms

✓ Transformation
✓ Transduction
✓ Conjugation
Direct uptake of exogenous genetic material

Griffith, Avery & MacLeod

Natural competence

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Bacillus spp.*
- *Neisseria spp.*

Induced competence

- Cold CaCl₂ & heat shock: *E. coli*
- Protoplast: *Bacillus, Streptomyces*
- Electroporation: a brief pulse of high-voltage electricity
Transfer of DNA by Bacteriophage via Lytic & Lysogenic cycle

Generalized transduction

Specialised transduction

Donor DNA integrates into cell chromosome

Combined DNA incorporates into chromosome

Adsorption

Recipient Cell

Virulent Phage

Donor Cell

Phage DNA

Viral DNA integrates into Chromosome

Cell Lyses

Transducing particle invades new cell

Transducing particles are assembled and the cell lysed

Phage

Bacterial Chromosome

Broken Down Bacterial Chromosome

Bacterial Chromosome is broken down
Conjugation

- Transfer of DNA from a donor to a recipient by direct physical contact between the cells
- Discovered 1946 Lederberg & Tatum

**Donor:**
- Containing conjugative plasmid (F+)

**Recipient:**
- Bacterial receiving the plasmid (F-)

*Image of conjugation process.*
Conjugation

High-frequency recombination (Hfr)

F' \rightarrow F^-

F' \rightarrow F^-

F' \rightarrow F^-

F' \rightarrow F^-

F' \rightarrow F^-

F' \rightarrow F^-
Genetic mechanisms of evolution of methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and MVRSA)
Defense against Transferred DNA

- Bacteria cut entering DNA to pieces
- Cut at specific restriction sites
- **Restriction enzymes**
  - Bacteria add methyl groups to DNA
  - Entering DNA is destroyed
  - Unless it comes from a similar species

EcoRI restriction/ modification site
- Heritable permanent change in DNA sequence
- Harmful, beneficial, or neutral
- Change in genotype
- May result in a change in phenotype
- Change is base sequence & failure to repair
Types of Mutations

- **Silent**
  - No effect on organism
  - Example: CGA → CGC (Arginine → Arginine)

- **Missense mutation**
  - Change one codon to another
  - Example: AAG → GAG ([lysine] → [glutamate])

- **Nonsense mutation**
  - Change a codon to Stop
  - Example: UAC → UAA (Tyrosine → STOP)

- **Frame shift mutation**
  - Insert or delete 1 or > nucleotides
  - Changes bases read by ribosome
  - Alters all codons downstream of mutation
  - Example: AAA CGA CCC → AAA CTG ACC C (Lysine Arginine Proline → Lysine Leucine Threonine)
AMES TEST

- Bruce Ames
- Reversion test
- Salmonella
- Mutation in hisG gene
- Expose to the mutagen
- Mutagen causes reversion
- Changes mutation to normal form
- Normally rare mutation
- More colonies = stronger mutagen