

Biology 6 Test 2 Study Guide

Chapter 6 – Microbial Growth and Culturing

A. Growth Requirements

a. Temperature

- i. All microbes have *optimal* growth temperature. It can grow up to *minimum* and *maximum* growth temps.
- ii. Types (Fig. 6.1)
 1. Psychrophiles – cold (range below 30 °C)
 2. Mesophiles – medium (37 °C optimum). Humans most concerned with these. Most common, can produce human toxins.
 3. Thermophiles – hot (range above 40 °C).

b. pH – same as temp except with acidity/alkalinity.

c. Osmotic pressure

- i. Specific salt concentration will change osmotic pressure. Tonicity (Fig. 6.4)
- ii. Most microbes live in low salt conditions. Halophiles live in high salt areas (e.g. Dead Sea/Great Salt Lake about 30%). Human is 1%, ocean is 3.5%.

d. Chemical

i. High quantities

1. Carbon – defines trophs (e.g. chemoautotrophs)
2. Nitrogen (can use ammonium NH_4^+ , nitrate NO_3^- , or nitrogen gas N_2)
3. Sulfur (hydrogen sulfide H_2S or sulfate SO_4^{2-})
4. Phosphorus (phosphate PO_4^{2-} only)
5. Oxygen
 - a. Required for some, harmful to others. Harmful because oxygen can turn into toxic forms: singlet oxygen gas, free radicals (e.g. O_2^- , H_2O_2 , OH)
 - b. Aerobes use oxygen, anaerobes do not. Ones that can tolerate oxygen must have enzymes to remove toxic forms. Know difference in growth of four types; obligate aerobe, facultative anaerobe, obligate anaerobe, microaerophile.

ii. Trace – needed in small amounts

1. Vitamins and minerals.

e. Culture Media

i. Chemical composition

1. Defined – exact chemical make up of medium is known (Tab. 6.3)
2. Complex – not defined. Usually made from extracts where exact composition is undetermined. (Tab. 6.4)

ii. Purpose (Tab. 6.5)

1. Selective – only allows growth of kind you want.
2. Differential – distinguishes colonies. (Fig. 6.9)
3. Enriched – allows growth of fastidious organisms.

iii. Special growth conditions – e.g. low oxygen or high carbon dioxide (Fig. 6.5-7)

iv. Rapid/Multitest Systems

1. Enterotube – solid media used for enteric (in your gut) organisms (Fig. 10.9)
2. Analytical Profile Index (API) – liquid media for enterics

B. Growth Dynamics

a. Cell division

- i. Occurs by binary fission in most prokaryotes. (Fig. 6.11)
 - ii. Generation time – time it takes to divide. Uninhibited growth is exponential. Best represented on a logarithmic scale. (Fig. 6.12, 13)
- b. Phases of growth in media (Fig. 6.14)
 - i. Lag phase – when first inoculated. Metabolic activity but no division yet.
 - ii. Log phase – growth is exponential.
 - iii. Stationary phase – growth is balanced by death or growth is simply arrested. Nutrients run out.
 - iv. Death phase – numbers decline in logarithmic fashion.
- c. Measurement techniques
 - i. Plate count
 - 1. Pour/spread plates are used to evenly spread culture onto plate (Fig. 6.17)
 - 2. Serial dilutions – for large quantities. CFU = # of colonies X dilution factor of that plate (Fig. 6.16)
 - 3. Filtration – for small quantities. Filter large volume to trap cells. Plate trapped cells. CFU = # of colonies/concentration factor.
 - ii. Microscopy – count known volume on cell counter. (Fig. 6.20)
 - iii. Spectrophotometry – measure turbidity by light absorbance. (Fig. 6.21)
 - iv. Metabolic activity and cell weight – products of metabolism (e.g. CO₂) or weight of cells can be taken.

Chapter 8 – Genetics and Molecular Biology

A. Overview

- a. Definitions
 - i. Genetics – study of heredity
 - ii. Genes – segments of DNA that code for functional products.
 - iii. Genotype – the genetic makeup
 - iv. Phenotype – physical trait
- b. Forms of DNA
 - i. Chromosomes – structures containing the cell's DNA. Also consists of protein.
 - ii. Plasmid – circular self-replicating pieces of DNA. Need host cell. (Fig. 8.1)
- c. Flow of genetic information - the Central Dogma: DNA → RNA → Protein (Fig. 8.2)

B. Replication

- a. Replication is semiconservative – half new and old. Template strand is parent strand that is being copied.
- b. DNA strands are antiparallel – runs in opposite direction (Fig. 8.3)
- c. Free nucleotides polymerize (Fig. 8.4)
- d. Replication begins at a replication bubble. Each side of the bubble is a replication fork (Fig. 8.6)
- e. Mechanism (Fig. 8.5)
 - i. Helicase unwinds DNA
 - ii. ssDNA binding proteins stabilize DNA
 - iii. Primase (RNA polymerase) makes small RNA primer that helps DNA polymerase make short fragments.
 - iv. DNA polymerase attaches nucleotides. Only 5' → 3'
 - 1. Leading strand is replicated continuously
 - 2. Lagging strand is replicated in pieces called Okazaki fragments

- v. DNA ligase connects pieces of DNA into one continuous strand
- C. Gene Expression Overview - Central Dogma: DNA → RNA → Protein (Fig. 8.2)
 - a. RNA
 - i. Uses AUGC
 - ii. mRNA, rRNA, tRNA
 - b. Genetic Code (Fig. 8.8)
 - i. Each triplet codes for one a.a.
 - ii. 64 possibilities with 20 a.a., therefore redundancy
 - iii. Note stop codons.
 - iv. Practice translation of sequence
- D. Transcription (Fig. 8.7)
 - a. 3 steps, initiation, elongation, termination
 - i. Initiation: RNA polymerase binds to promoter. DNA strands are separated.
 - ii. Elongation: Template strand is read and new RNA is made.
 - iii. Termination: A termination signal is reached and RNA polymerase dissociates.
 - b. Eukaryotes (Fig. 8.11)
 - i. Additional splicing: introns removed, exons kept.
 - ii. Exons are exported from nucleus to find ribosome.
- E. Translation (Fig. 8.9)
 - a. Ribosome is enzyme: has a small and large subunit.
 - b. tRNA: one for each a.a. Becomes charged with a.a. Has anticodon that is complementary to mRNA.
 - c. 3 Steps
 - i. Initiation: small ribosome and first tRNA binds mRNA. Large subunit comes on top.
 - ii. Elongation: new aa-tRNA comes in to empty site. Polymerization, shift of ribosome. Used tRNA exits.
 - iii. Termination: When hits stop codon, no tRNA (sometimes a release factor). Complex falls apart.
 - d. Prokaryotes can do coupled transcription-translation (Fig. 8.10)
- F. Regulation of Bacterial Gene Expression
 - a. Types
 - i. Activation – an activator turns on transcription
 - ii. Repression – a repressor blocks transcription. An inducer removes repressor.
 - b. Lac Operon (Fig. 8.12)
 - i. Background: bacteria prefer to use glucose over lactose as carbon source. However, if lactose is present and glucose is not, it will use it. Three genes are necessary to use lactose: Z, Y, A. These only need to be turned on when lactose is present and glucose is absent.
 - ii. Repression: The O site (operator) is bound by *I* protein. This turns off genes by blocking RNA polymerase. When lactose is present, it will bind *I* and pull it off.
 - iii. Activation: When glucose is low, cAMP is high in cell. cAMP binds CAP and together act as a coactivator. They bind AS and recruit RNA polymerase to the promoter.
- G. Mutations
 - a. Types (Fig. 8.17)
 - i. Deletion – remove bases
 - ii. Insertion – insert bases
 - iii. May result in frameshift mutation (must be other than multiple of 3 a.a.)
 - iv. Point mutation – base mismatch substitution.
 - v. Consequences of mutations

1. Silent – a.a. is unchanged
 2. Missense – a.a. is changed. Can be harmful or neutral.
 3. Nonsense – a.a. changed to stop.
- vi. Practice mutations with genetic code.
- b. Causes
- i. Replication mechanism – DNA polymerase makes mistakes $1/10^3$ to $1/10^9$ bases.
 - ii. Chemicals
 1. May cause modification of a base to cause mispairing. (Fig. 8.18)
 2. May cause small insertions or deletions. E.g. soot or other compounds can sit in between bases and force a gap.
 - iii. Ionizing radiation – rays will ionize normal compounds and make them react inappropriately with other molecules. E.g. form covalent bonds.
 - iv. Ultra violet light (UV) – forms T-T dimers. These stall replication
- c. Repair
- i. Repair mechanisms exist to fix mistakes (Fig. 8.20)
 - ii. DNA polymerase can repair its own mistakes to a mutation rate of $1/10^9$.
- d. Frequency – every replication gives $1/10^9$ rate of mistakes.
- i. E. coli has 4.6 million bp. This is about 1 mistake in 250 cells replicated.
 - ii. Each gene has about 1000 bp and with $1/10^9$ mistakes, $1/10^6$ chance a gene will be mutated every replication.
 - iii. Theory is that mistakes are allowed for evolution to occur.
- e. Creating and selecting mutants
- i. Negative selection – the mutant is killed off in selection due to loss of trait. Need to replica plate to find mutant. (Fig. 8.21)
 - ii. Positive selection – kill off cells that did not mutate. E.g. Ames Test gives rate of mutation based on ability to gain a trait. E.g. His- to his+ (Fig. 8.22)

H. Gene Transfer

- a. Mechanism – recombination. DNA can exchange across strands as long as there is sequence identity
- b. Types of Gene Transfer
 - i. Transformation – naked DNA taken into cells
 1. Griffith 1928 first demonstrated transformation. (Fig. 8.24)
 - a. S strain kills mice, R strain does not kill. R + heat-killed S kills mice.
 - b. This “transforming principle” was later discovered to be DNA
 2. Used experimentally (Fig. 8.25)
 - a. Bacteria can be made competent by treatment to loosen cell wall and membrane.
 - b. Genes will recombine into chromosome or plasmid.
 - ii. Conjugation – sex! DNA is transferred directly from one cell to another. Mediated by F factor. (Fig. 8.26, 8.27)
 1. Lederberg 1949 showed conjugation by complementation of traits.
 2. F factor plasmid transfer: F+ cell mates with F- cell and converts it to F+. If integration of F factor occurs, new cell is Hfr (high freq. recombination)
 3. Hfr transfer: Hfr mates with F- and transfers only part of DNA. Recipient is still F-. F' can occur from Hfr by excision. Now it acts just like an F factor plasmid.
 - iii. Transduction – infection by virus (Fig. 8.28)
 1. Bacteriophage is a virus of bacteria

2. Virus infects by attaching to a cell and injecting DNA. DNA is expressed and new virus particles are created. Host DNA is degraded. Newly formed viruses can take host DNA fragments to another cell.
- iv. Transposons – jumping genes (Fig. 8.30)
 1. Mobile segments of DNA.
 2. Minimum elements
 - a. Transposase gene to facilitate recombination. Can cut and paste DNA strands.
 - b. Inverted repeats – sequences that target new location and also is recognized by transposase.

Chapter 9 - Biotechnology

A. Biotechnology

- a. Recombinant DNA technology – genes mixed from different organisms.
 - i. Create new strains, or produce a product (Fig. 9.1)
 - ii. Restriction enzyme cloning (Fig. 9.2)
 1. Restriction enzymes cut DNA at specific sites. Can produce “sticky ends” that can base pair to other sticky ends. (Tab 9.1)
 2. DNA ligase covalently binds the strand.
 3. Transform into bacteria and select colonies.
- b. PCR-polymerase chain reaction. For amplification of specific sequence. (Fig. 9.4)
 - i. 3 steps:
 1. Denaturation (94 °C) – Separate DNA strands.
 2. Annealing (60 °C) – Primers bind to DNA.
 3. Polymerization (72 °C) – A thermophilic DNA polymerase polymerizes new strand
 - ii. Each cycle doubles amount
- c. Gel electrophoresis
 - i. Separates DNA fragments by size and makes them visible.
 - ii. DNA migrates through a gel towards positive charge.
 - iii. Can be used in DNA fingerprinting.
- d. Cell Fusion
 - i. Protoplast fusion – remove cell walls and mix two cell types. (Fig. 9.5)
 - ii. Hybridomas – two different types of animal cells fused together. E.g. myeloma and B cell grows easily and produces antibodies

B. Applications

- a. Therapies
 - i. Vaccine production
 - ii. Gene therapy – cells removed from body, repaired, returned.
- b. Forensics (Fig. 9.17)
- c. Drug factory – bacteria, yeast, plants and animals can be used to make human therapeutics
- d. Agriculture – improve crops and livestock: disease resistance, increase yield (Fig. 9.20)
- e. Ethics
 - i. Safety of GM foods
 - ii. Screening for insurance companies
 1. Sickle-cell screening caused discrimination in military.
 2. Tay-Sachs screening helped reduce disease.
 - iii. Cloning humans.