GROWTH, CONTROL,
CHEMOTHERAPY
GROWTH, CONTROL, CHEMOTHERAPY

GROWTH

Binary Fission, Growth Rate, Generation Time, $N = N_0 2^t$
Growth Phases of Culture - Lag, Exponential, Stationary, Death
Physical Factors - Temperature - psychrophile, mesophile, thermophile
                           hypertermophile
                           $\text{pH, Buffer, acidophile, alkalophile}$
Osmotic Pressure, halophile
Oxygen, Aerobic, Anaerobic

CONTROL OF MICROBIAL GROWTH

Sterilization by Heat, Pasteurization
Sterilization by Filtration
Antimicrobial Agents - bacteriocidal, bacteriostatic
                           Disinfectants, Antiseptics

CHEMOTHERAPEUTIC AGENTS

Enrich - Salvarsan - Selective Toxicity

Doxaz - Sulfonamides - Sulfa Drugs
                           Sulfanilamide - $p$-Aminobenzoic Acid - Folic Acid Synthesis Inhibitor

Pamming - Penicillin - Peptidoglycan Synthetase Inhibitor
                           $\text{Penicillium notatum}$

Waksman - Streptomycin, $\text{Streptomyces griseus}$
                           $\text{Mycobacterium tuberculosis}$
                           Protein synthesis inhibitor (70S ribosomes)

Antiviral Agents
                           Azidothymidine (AZT) - antiretroviral agent
                           Interferon - species-specific antiviral protein
NUMBER OF GENERATIONS AND GENERATION TIME

\[ n = \frac{t}{g} \]

\[ n = \text{NUMBER OF GENERATIONS} \]

\[ t = \text{TIME IN HOURS} \]

\[ g = \text{GENERATION TIME IN HOURS/GENERATION} \]

\[ n = 10 \text{ HOURS/} 0.5 \text{ HOURS/GENERATION} = 20 \text{ GENERATIONS} \]

\[ g = 10 \text{ HOURS/} 20 \text{ GENERATIONS} = 0.5 \text{ HOURS/GENERATION} \]

\[ g = \frac{t}{n} \]
OXYGEN

AEROBIC - O$_2$ TERMINAL ELECTRON ACCEPTOR
ANAEROBIC - SOMETHING OTHER THAN O$_2$

AEROBIC, OBLIGATE - REQUIRE O$_2$
EX, PSEUDOMONAS AERUGINOSA

MICROAEROPHILIC - REQUIRE O$_2$, BUT IN LOW CONCENTRATION
EX, NEISSERIA GONORRHOEAE

FACULTATIVE ANAEROBIC GROW WITH OR WITHOUT O$_2$; BETTER WITH O$_2$
EX, ESCHERICHIA COLI

AEROTOLERANT ANAEROBIC GROW EQUALLY WELL WITH OR WITHOUT O$_2$
EX, ENTEROCOCCUS FECALIS

OBLIGATE CANNOT GROW IN O$_2$
ANAEROBIC
EX, CLOSTRIDIUM TETANI
\[ \text{pH} = - \log_{10} [H^+] \] CONCENTRATION IN MOLES/LITER

\[ H_2O \rightleftharpoons H^+ + OH^- \]

\[ [H^+] = 1 \times 10^{-7} \text{ MOLAR} \]
\[ [OH^-] = 1 \times 10^{-7} \text{ M} \]

\[ K_{eq} = \frac{[H^+] \times [OH^-]}{[H_2O]} \]

\[ [H_2O] = 55 \text{ M} - \text{VERY BIG} \]

SIMPLIFY: \[ K_w = [H^+] \times [OH^-] = [1 \times 10^{-7}] \times [1 \times 10^{-7}] \]
\[ = 1 \times 10^{-14} \]

\[ \text{pH} = - \log_{10} 1 \times 10^{-7} = -(-7) = 7 \]
Because \([H^+] \times [OH^-] = 1 \times 10^{-14}\), we can determine \([OH^-]\).
Typical Bacterial Cell-Binary Fission

0 min

1 x 3 μm

20

40

60 min
Binary Fission

Gram Negative Bacillus

CM

Outer Membrane Peptidoglycan

New Peptidoglycan
Binary Fission

Gram Negative Bacillus

NUCLEOID

- Outer Membrane
  - Peptido-Glycan
  - Cytoplasmic Membrane

New Synthesis of Outer Membrane Peptido-Glycan Cytoplasmic Membrane DNA (Nucleoid)
DEALING WITH NUMBER OF BACTERIA

\[ g = \text{HOURS PER GENERATION} \]

\[ \text{EX, 0.5 HR/GENERATION, CELLS DIVIDE EVERY 30 MINUTES} \]

\[ N = N_0 2^n \]

\[ N = \text{NUMBER OF BACTERIA AFTER } n \text{ GENERATIONS} \]

\[ N_0 = \text{INITIAL NUMBER OF BACTERIA} \]

\[ n = \text{TOTAL NUMBER OF GENERATIONS} \]

EXAMPLE: CALCULATE \( N \), IF \( N_0 = 1,000 \) AND \( n = 5 \)

\[ N(\text{AFTER 5 GENERATIONS}) = 1,000 \times 2^5 = 1,000 \times 32 = 32,000 \]

USUALLY MEASURE CELLS/UNIT VOLUME:

\[ N(\text{CELLS/ML}) = 1,000(\text{CELLS/ML}) \times 2^5 = 32,000 \text{ CELLS/ML} \]
Penicillin - Fleming - 1929

Penicillium notatum

Florey - purified penicillin

Nobel

$\beta$ lactam ring

$R = \text{CH}_2$ - natural penicillin G
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GROWTH IN LIQUID

LOG_{10} LIVING CELLS /ML

LAG

EXPONENTIAL

STATIONARY

DEATH

~ 12 HRS

TIME

INOCULUM = STARTING CELLS

ABSORBANCE TURBIDITY
PHYSICAL MEANS OF MICROBIAL CONTROL

STERILIZATION - COMPLETE KILLING

AUTOCLAVE - 121 °C, 15 PSI, 15 - 30 MIN

PASTEURIZATION - KILLS MOST PATHOGENS

63 - 66 °C 30 MINUTES

HTST - HIGH TEMPERATURE, SHORT TIME FLASH PASTEURIZATION

74 °C 15 SECONDS

UHT - ULTRA HIGH TEMPERATURE

140 °C < 1 SECOND
STABLE, ROOM TEMP 30 DAYS

DESSICATION - DRYING

KILLS HIV, NEISSERIA GONORRHOEAE

SURVIVE: MYCOBACTERIUM, ENDOSPORES, MOLD SPORES
HIGH OSMOTIC PRESSURE - SALT, SUGAR

RADIATION - UV, X-RAYS, GAMMA RAYS
MUTATIONS, CARCINOGENS

FILTRATION - STERILE MEMBRANE
0.45 MICRON (MICROMETER) DIAMETER
PORE

CHEMICAL CONTROL

DISINFECTION - REMOVE OR KILL MOST
PATHOGENS ON INANIMATE OBJECTS,
DISINFECTANT

ANTISEPTIC - CHEMICAL APPLIED TO BODY TO
KILL MOST PATHOGENS

BACTERICIDAL - KILLING BACTERIA

BACTERIOSTATIC - PREVENTING BACTERIAL
GROWTH
ACIDS - FOOD ADDITIVES, PROPIONIC ACID, CALCIUM PROPIONATE, GLUTAMIC ACID, MONOSODIUM GLUTAMATE

ALCOHOLS - ISOPROPYL ALCOHOL, ETHYL ALCOHOL, ANTISEPTICS, ALCOHOLS DENATURE PROTEINS

PHENOLICS - PHENOL - LISTER PHENOL DERIVATIVES: HEXACHLOROPHENE, LYSOL

HALOGENS - CHLORINE - BLEACH

\[ \text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{Cl}^- + \text{HOCl} \]

(HYPOCHLOROUS ACID)

IODINE - TINCTURE OF IODINE = 2% SOLUTION IN ALCOHOL, ANTISEPTIC IODINE TABLETS FOR WATER PURIFI.

ISODINE = ORGANIC IODINE DERIVATIVE
HEAVY METALS - ENZYME POISONS
SILVER NITRATE - AgNO₃

ANTISEPTIC AGAINST *NEISSERIA GONORRHOEAE*

MERCURY, Hg, ORGANIC DERIVATIVES:
MERTHIOLATE,
MERCUROCHROME

HYDROGEN PEROXIDE, H₂O₂, WOUND CLEANING

ETHYLENE OXIDE - POISONOUS GAS,

FORMALDEHYDE - TISSUE PRESERVATIVE
CHEMOTHERAPY - DRUG TREATMENT, SELECTIVE TOXICITY

EHRLICH - 1906 SYPHILIS CURE
SALVARSAN, ARSENIC DERIVATIVE
TREPONEMA PALLIDUM
COINED TERM: CHEMOTHERAPY

1930s - SULFA DRUGS, SULFONAMIDES
ANTIBACTERIAL, STRUCTURAL ANALOGS
OF PARA AMINO BENZOIC ACID
PARA AMINO BENZOIC ACID

FOLIC ACID
VITAMIN NUCLEIC ACID SYN.
SOME AMINO ACIDS

SULFANILAMIDE

REACTS WITH AND INHIBITS ONE ENZYME OF FOLIC ACID SYNTHESIS

WHY SELECT ME?
STRUCTURE OF PENICILLIN
[A β-LACTAM]

β LACTAM RING

AN INHIBITOR OF TRANS PEPTIDATION IN PEPTIDOGLYCAN CROSS LINKING -
A STRUCUTRAL ANALOG OF D-ALANINE - D-ALANINE
Cross Link Bond Will Be Formed

Transpeptidation → D-Alanine

Penicillin Inhibits - It is a Structural Analog of D-Ala-D-Ala
STREPTOMYCIN - WAKSMAN, 1945

STREPTOMYCES GRISEUS

WAKSMAN COINED TERM ANTIBIOTIC: SOMETHING PRODUCED BY ONE ORGANISM WHICH IS TOXIC FOR ANOTHER ORGANISM

PROTEIN SYNTHESIS INHIBITOR

INHIBITS PROKARYOTIC RIBOSOMES (70S)

FIRST CURE FOR MYCOBACTERIUM TUBERCULOSIS
MECHANISM OF ACTION

1. CELL WALL SYNTHESIS INHIBITION E.G. PENICILLIN

2. DISRUPTION OF CYTOPLASMIC MEMBRANE FUNCTION E.G., POLYMIXIN

3. PROTEIN SYNTHESIS INHIBITION E.G. STREPTIMYCIN TETRACYCLINE ERYTHROMYCINE

4. NUCLEIC ACID SYNTHESIS INHIBITION RNA – RIFAMYCIN DNA – NOVOBIOCIN

5. ACTION AS ANTIMITABOLITE E.G. SULFA DRUGS
ANTIMETABOLITES – SUBSTANCES THAT PREVENT A CELL FROM CARRYING OUT METABOLIC REACTION = MOLECULAR MIMICRY

ANTIMETABOLITES FUNCTION TWO WAYS:

1. BY COMPETITIVE INHIBITION OF ENZYMES [SULFONAMIDES] [ENZYME IS INHIBITED BY A SUBSTRATE WHICH BINDS, BUT CAN NOT REACT]

2. BY BEING INCORPORATED [BECAUSE OF SIMILAR 3-DIMENSIONAL SHAPE] INTO IMPORTANT MOLECULES SUCH AS NUCLEIC ACID. [AZT]
SIDE EFFECTS

TOXICITY - STREPTOMYCIN - OTIC NERVE DAMAGE
- AZIDOTHYMIDINE (AZT) - LYMPHOCYTE DEVELOPMENT

ALLERGY - ANAPHYLAXIS
PENICILLIN HYPERSENSITIVITY

NORMAL FLORA DESTRUCTION - CANDIDA ALBICANS CLOSTRIDIUM DIFFICILE

SELECTION OF DRUG - RESISTANT PATHOGEN MUTANTS
- PENICILLIN-RESISTANT NEISSERIA GONORRHOEAE SPECTINOMYCIN; CEFTRIAXONE
- AZT-RESISTANT HIV
HIV DNA SYNTHESIS FROM RNA

SINGLE-STRAND RNA

REVERSE TRANSCRIPTASE

+ LOW MOLECULAR WEIGHT DNA PRECURSORS

RNA DNA

RNA DEGRADATION

2ND DNA STRAND SYNTHESIS
HIV DNA synthesis inhibition by AZT

Single strand RNA

Reverse transcriptase

+ AZT [structural analog]
+ low molecular weight precursors

Growing DNA strand contains AZT - it can not be extended farther.