Microbes and Maths
J-Term 2022

NORTH CAROLINA SCHOOL OF SCIENCE AND MATHEMATICS

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Introduction to Microbiology

Microbial growth

Introduction to the experimental design

Introduction to Microbiology

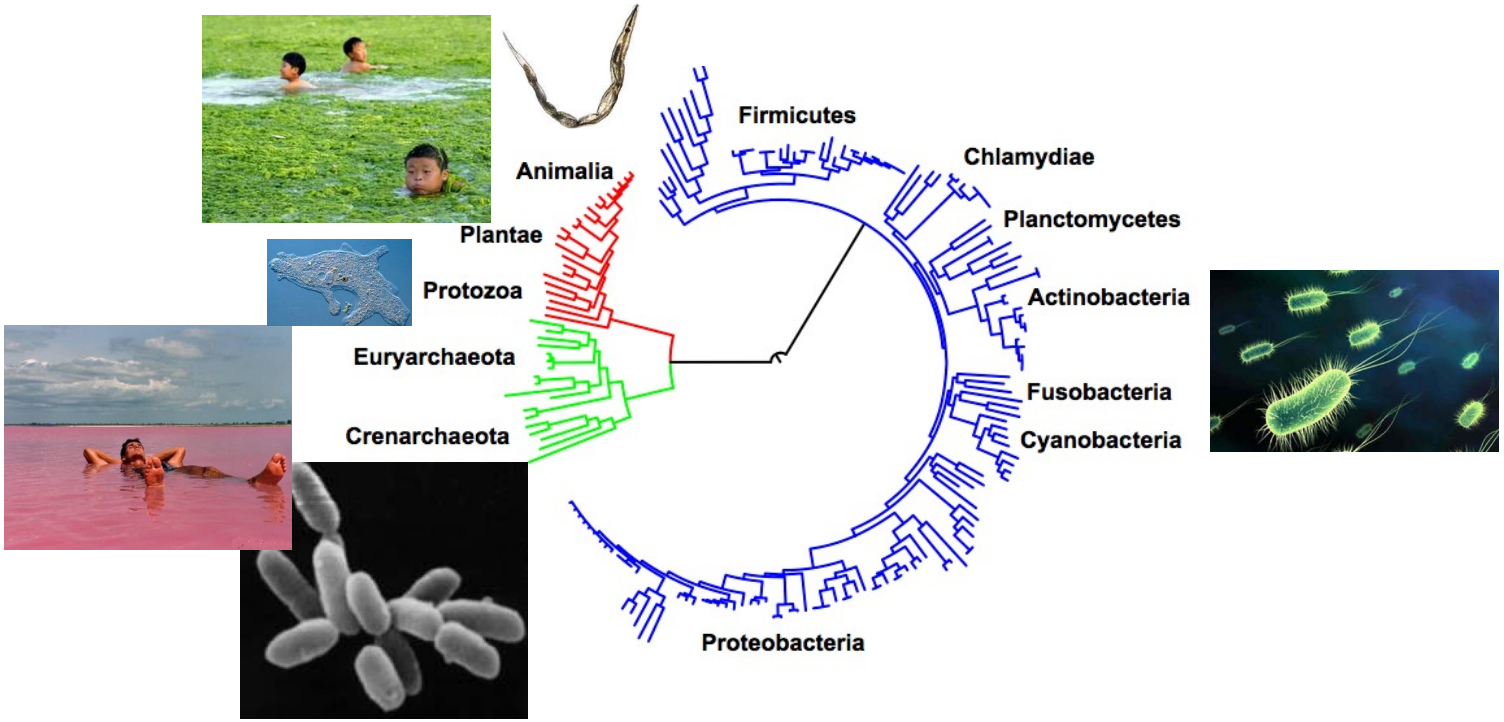
INTRODUCTION

DEFINITION:

Microbiology. The study of microorganisms.

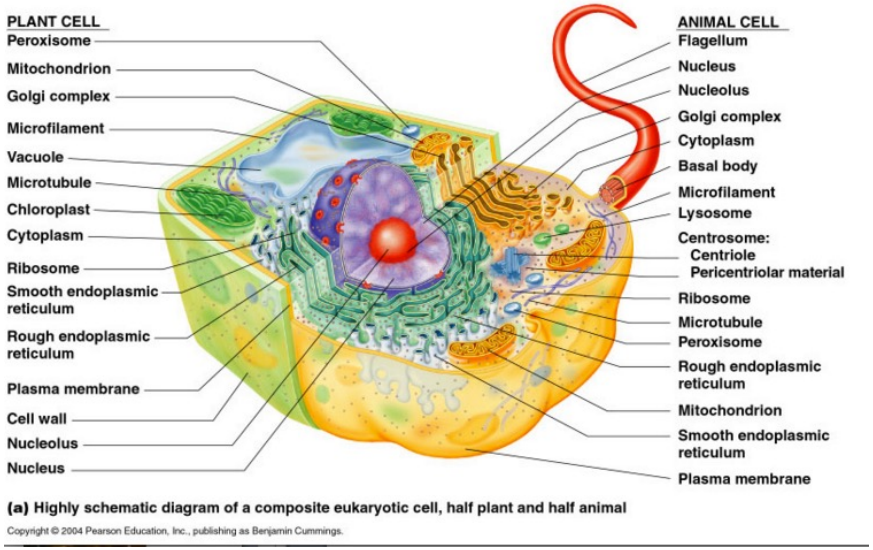
Microorganism. Live organisms that are invisible to the naked eye.

CLASSIFICATION:



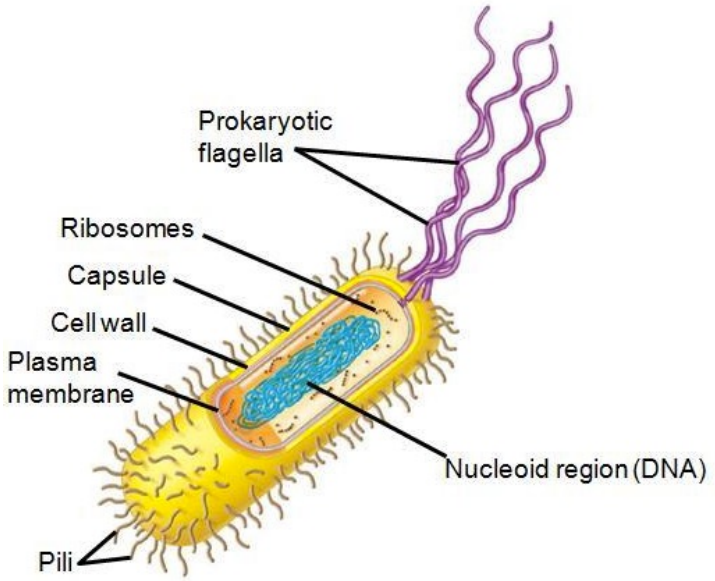
INTRODUCTION

Eukaryote:



(a) Highly schematic diagram of a composite eukaryotic cell, half plant and half animal

Prokaryote:



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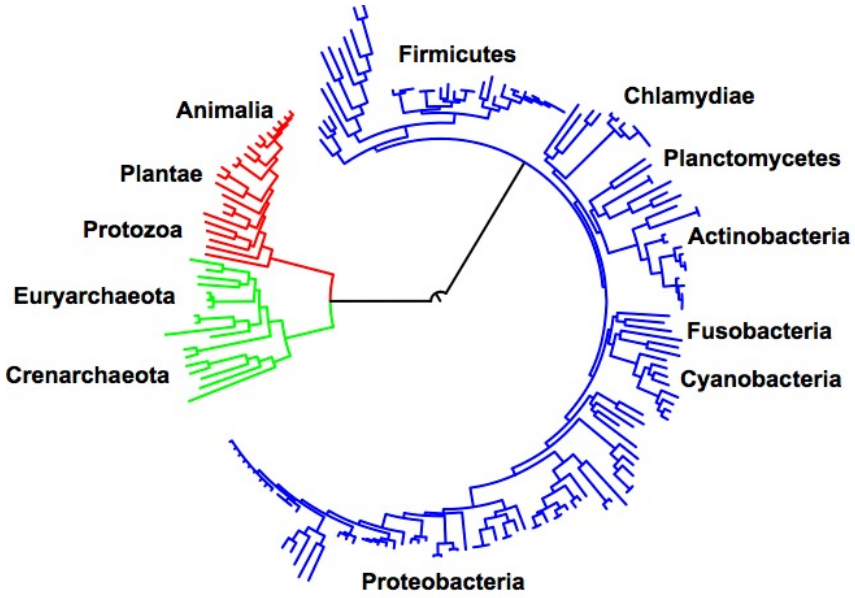
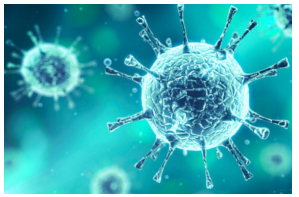


Viruses



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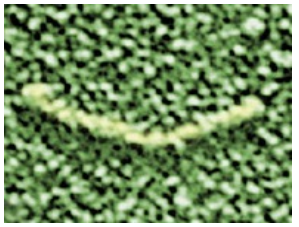
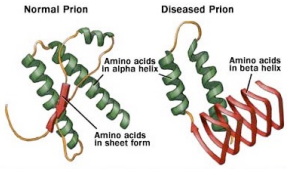
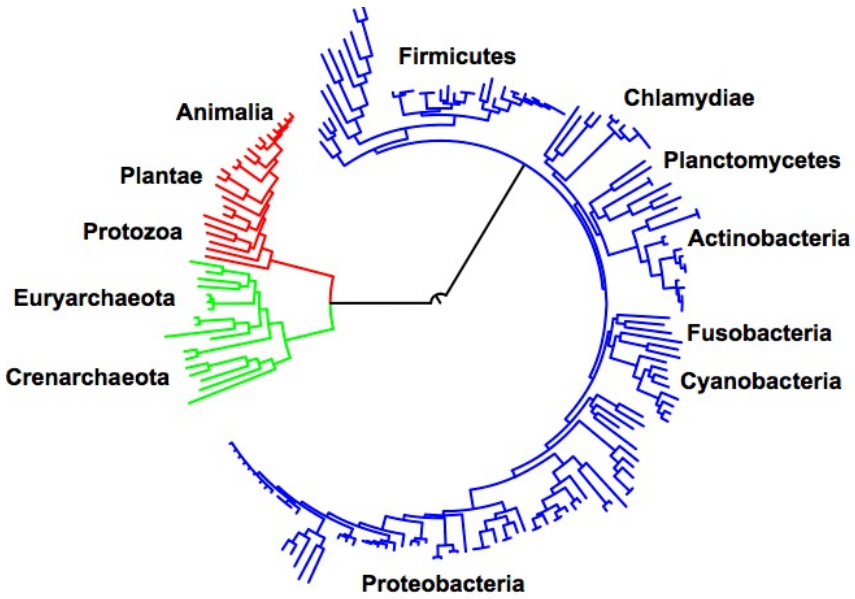
Viroids



Prions



INTRODUCTION



Eukaryote: Organism unicelullar or multicelular whose cells contain a nucleus and other organelles enclosed with membranes.

Prokaryote: Single-celled organisms that lacks a membrane-bound organelles.

Archaea: organisms that constitute a different kingdom in the life tree. They are prokaryotic however, they are closer to eukaryotes in terms of evolution.

Protozoa: Unicellular eukaryotic organisms. They are 'plant-like" as they are able to obtain energy from light (photosynthesis) but have "animal-like" behaviors, such as motility or predation.

Virus: Infectious agent that replicates inside living cells.

Viroid: Infectious agent that consist of short single-stranded RNA (without protein coat) and infect plants.

Prion: Infectious agent that consist in misfolding proteins that can be transmited in between organisms.

INTRODUCTION

Protozoa:

1-10 µM

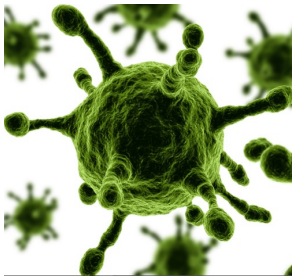
Bacteria:

0.3-5 µM

Archaea:

1 µM

Virus:

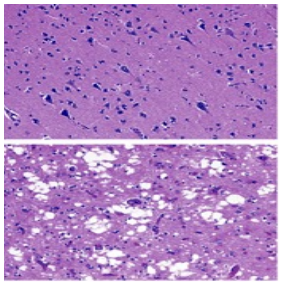


20-500 nM

Viroid:

Measured in nucleobases

Prion:

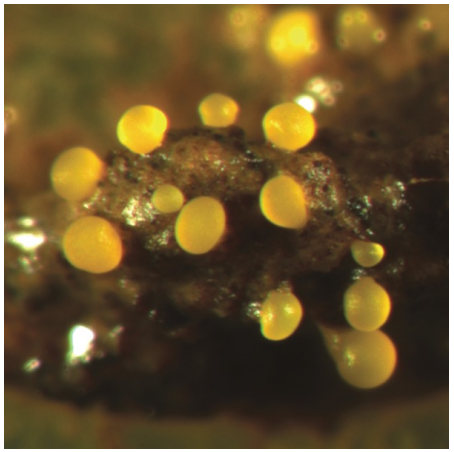


Measured in KDa

Nacked eye can not see under 20-30 µM!!!

WHAT ROLE DO MICROORGANISMS PLAY IN THE ENVIRONMENT?

- Oxygen production!
  - cyanobacteria in the ocean
- Nitrogen fixation
  - symbionts with many plants
- Decompose dead material
- Help animals digest food
- Decontamination

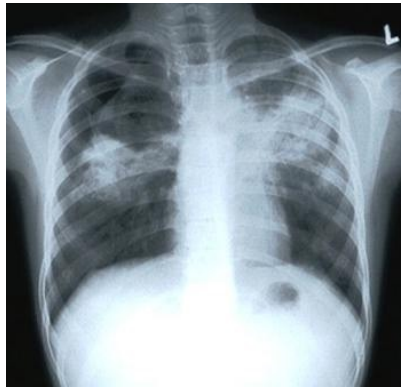


HOW DO WE USE MICROORGANISMS?

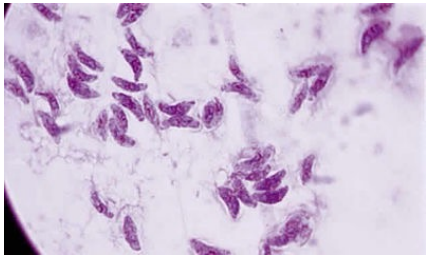
Product	Microorganism
Cheese	bacteria, fungi and milk
Alcoholic beverages	bacteria, yeast and sugar
Soy sauce	fungi and soybeans
Vinegar	bacteria and sugar
Yogurt	bacteria and milk
Sour cream	bacteria and cream
Artificial sweetener	bacteria and sugar
Bread	yeast and dough
Antibiotics	bacteria, fungi
Human Growth Hormone, human Insulin	genetically engineered bacteria
laundry enzymes	bacteria
vitamins	bacteria

HOW DO MICROORGANISMS USE US?

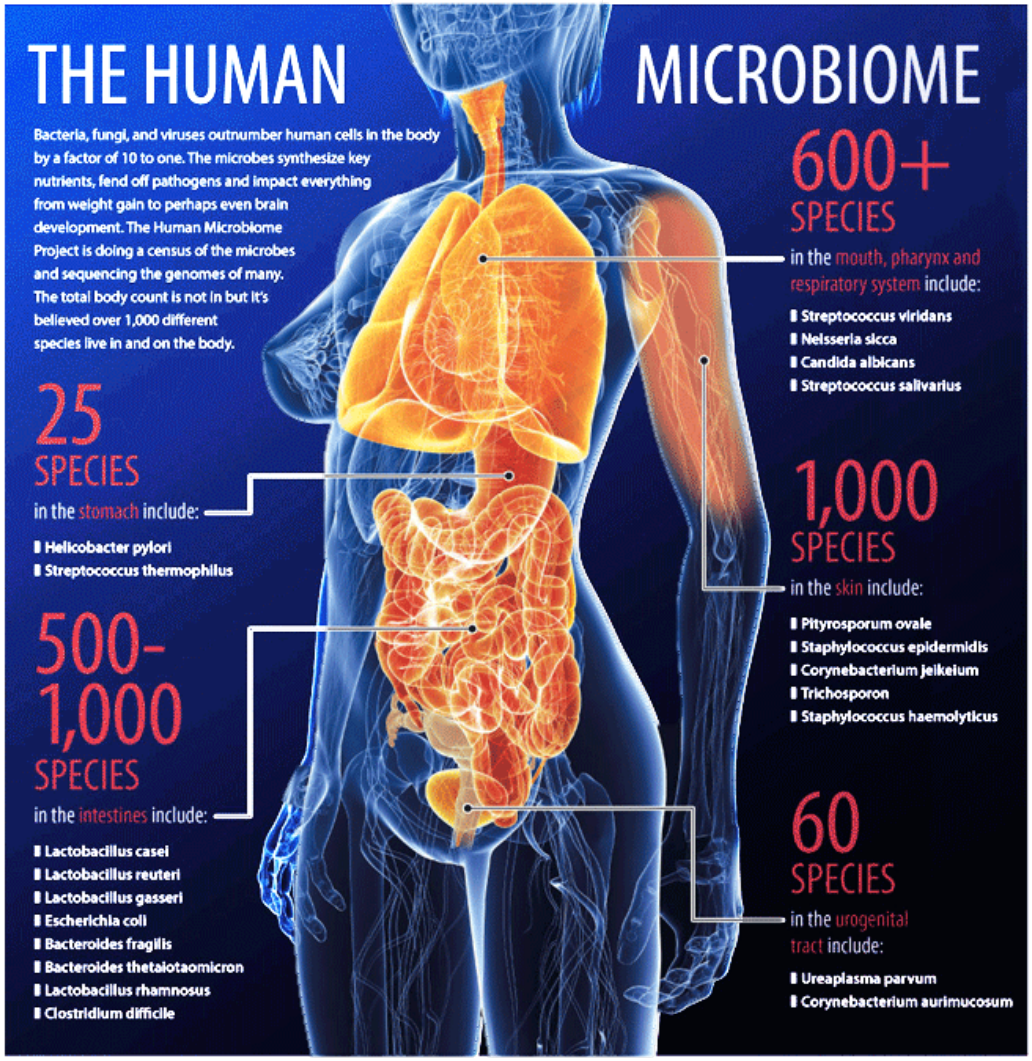
- MRSA
  - Methicillin Resistant *Staphylococcus aureus*
- VRE
  - Vancomycin Resistant *Enterococcus*
- VRSA
  - Vancomycin Resistant *Staphylococcus aureus*
- MDR-TB
  - Multidrug Resistant Tuberculosis



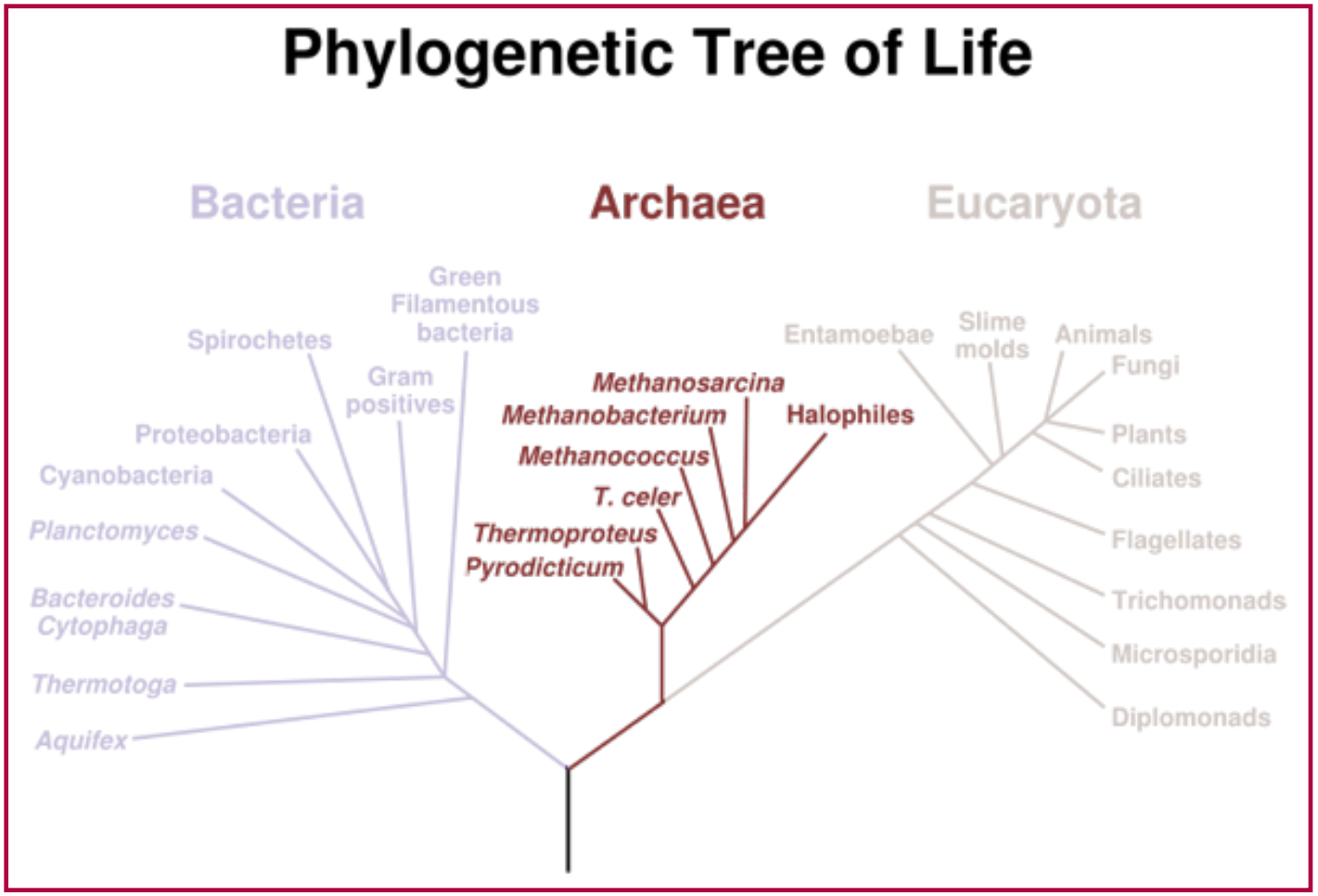
- Malaria
  - *Plasmodium* parasite
- Toxoplasmosis
  - *Toxoplasma* parasite
- Zika virus
- Ebolavirus



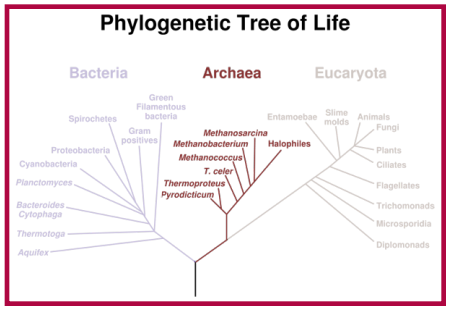
MICROBIOTA vs MICROBIOME

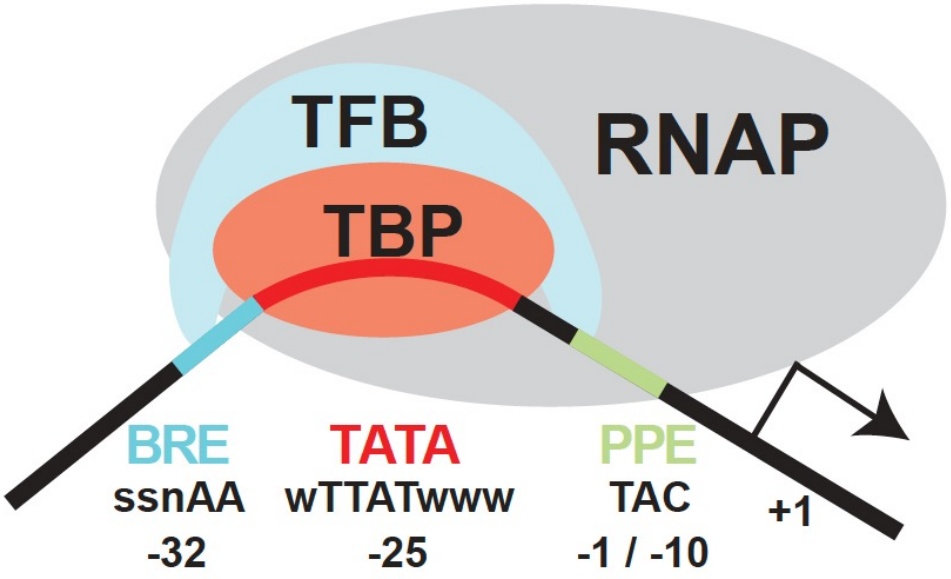


Archaea as a model organism



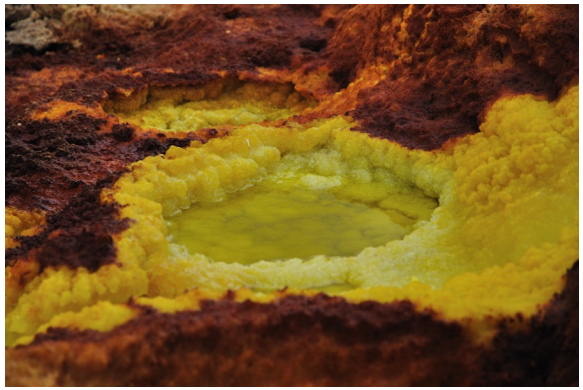
Archaea as a model organism





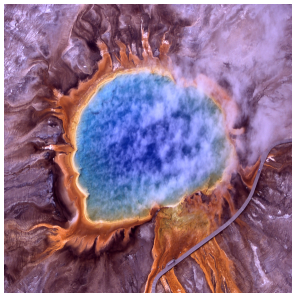
EXTREMOPHILE

Extremophiles are organisms that thrive in very extreme environments - they can survive conditions that would kill most other organisms!

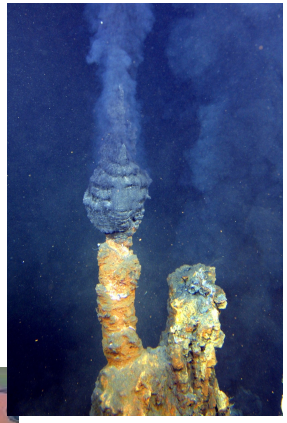


TYPES OF EXTREMOPHILES

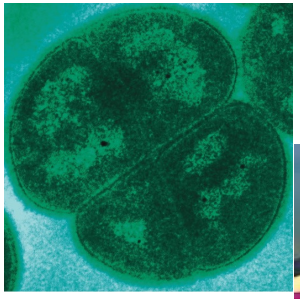
THERMOPHILES/PSYCHROPHILES



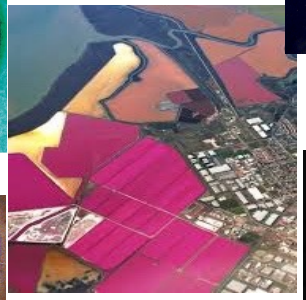
ACIDOPHILES/ALKALIPHILES



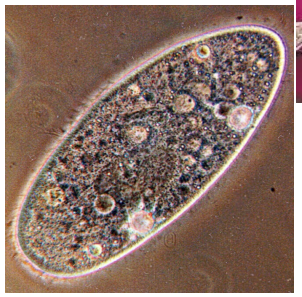
BAROPHILES



RADIOPHILES

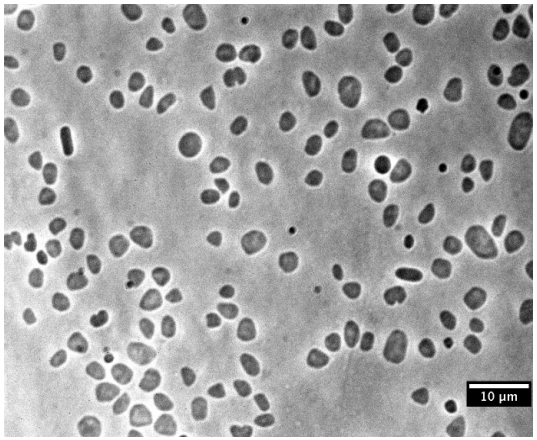


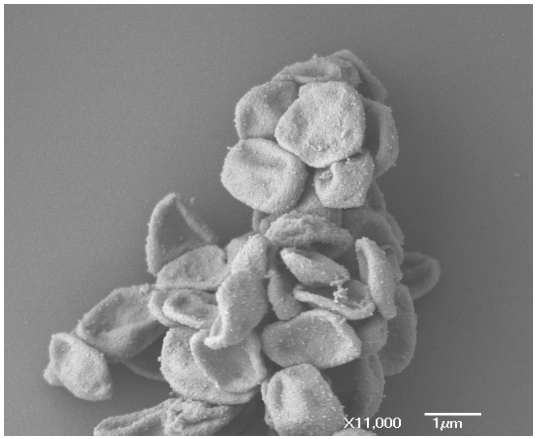
HALOPHILES



XEROPHILES

Haloferax volcanii AS A MODEL ORGANISM





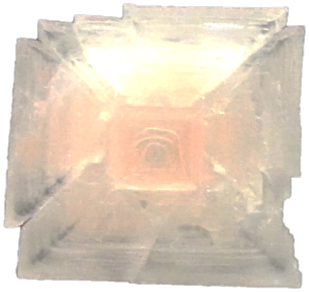
Description and significance
halophilic
mesophile
isolated from hypersaline environments such as: the Dead Sea, the Great Salt Lake, and oceanic environments.Extremophile.
H. volcanii is chemoorganotrophic, metabolizing sugars as a carbon source.
It is primarily aerobic, but is capable of anaerobic respiration under anoxic conditions.[

Genome structure
The genome of *H. volcanii* consists of a large (4 Mb), multicopy chromosome and several megaplasmids.
The complete genome, DS2, of *H. volcanii* consists of about 4130 genes.[3]
The genome has been completely sequenced.

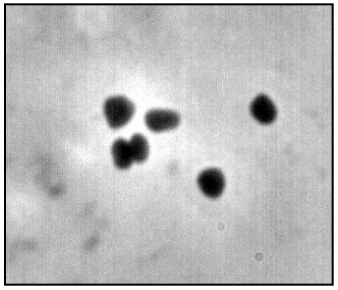
Cell structure and metabolism
Optimal grow at 42 ° C in 1.5-2.5 M NaCl and complex nutrient medium.

Haloferax volcanii AS A MODEL ORGANISM

Fun facts about *Haloferax volcanii*

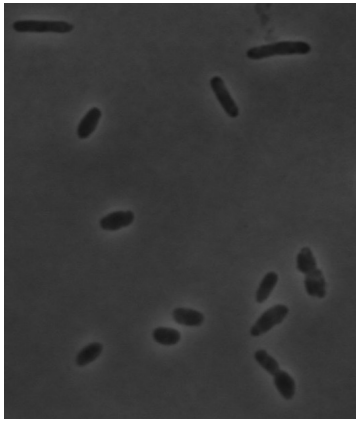


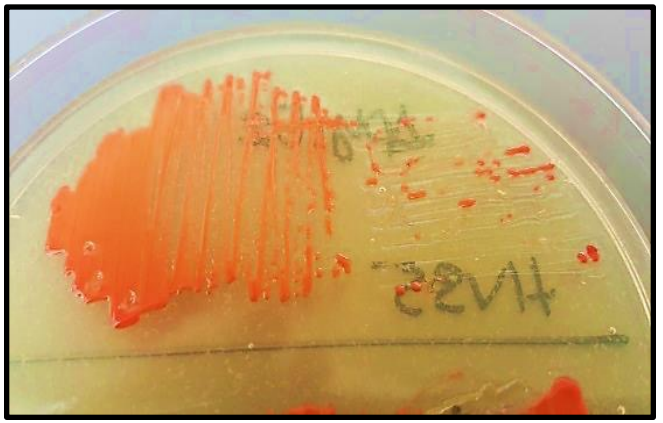
•Prefers 2.5 M NaCl, 45° C for optimal growth
•Isolated from the Dead Sea
•Grows fast



Not fun facts about *Haloferax volcanii*

•Named for microbiologist Benjamin Elazari Volcani and has nothing to do with a volcano.
•Smelly





TAXONOMY

The study of **phylogenetic** relationships between organisms

DOMAIN Three domains of life
KINDOM related phylum
PHYLUM related classes
CLASS related orders
ORDER related families
FAMILY related genera
GENUS closely related species
SPECIES organisms sharing a set of biological traits and reproducing only with their exact kind

Further classifications especially with bacteria:
Strain—organisms within a species varying in a given quality
Type—organisms within a species varying immunologically

Introduction to Microbiology

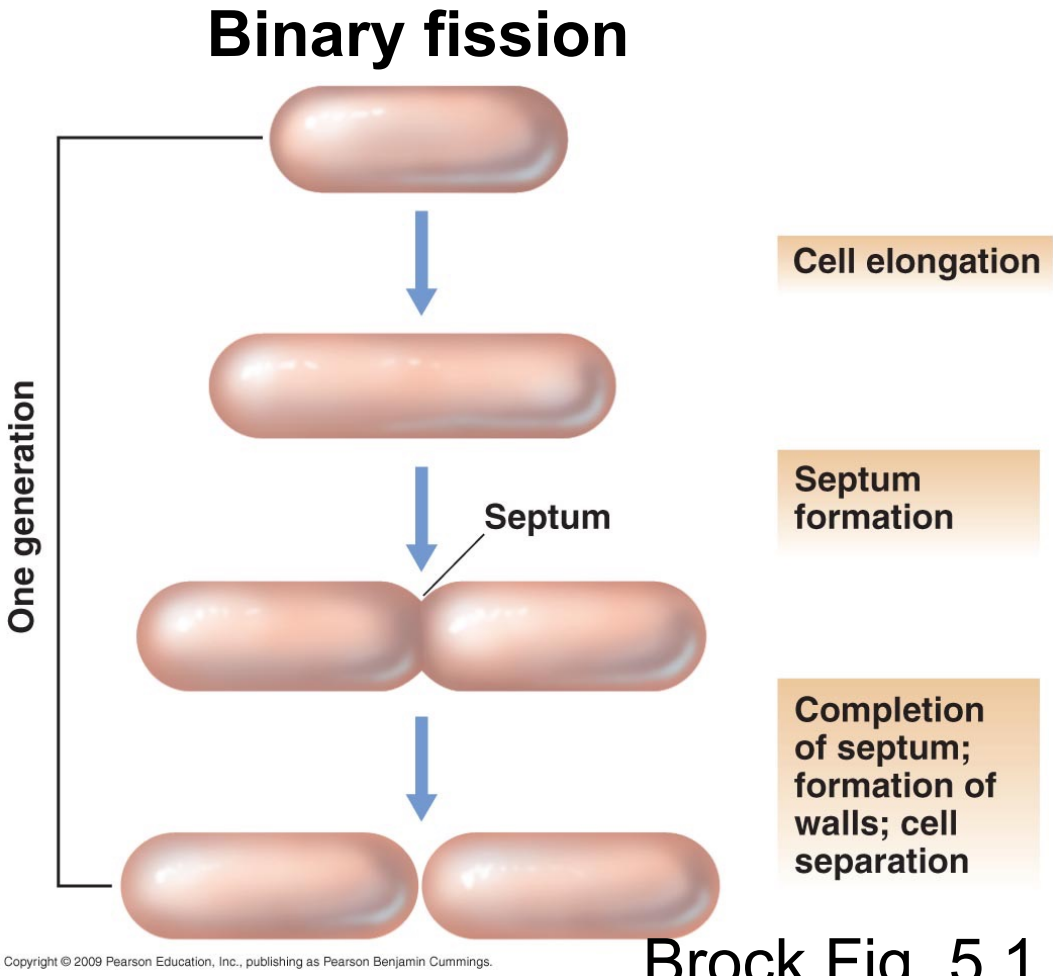
Microbial growth

Introduction to the experimental design

Microbial growth

Bacterial growth:

Binary fission: conventional bacteria



Brock Fig. 5.1

Yeast growth:

Buding (yeast, but also in bacteria) the buding cell is smaller than mother cell.

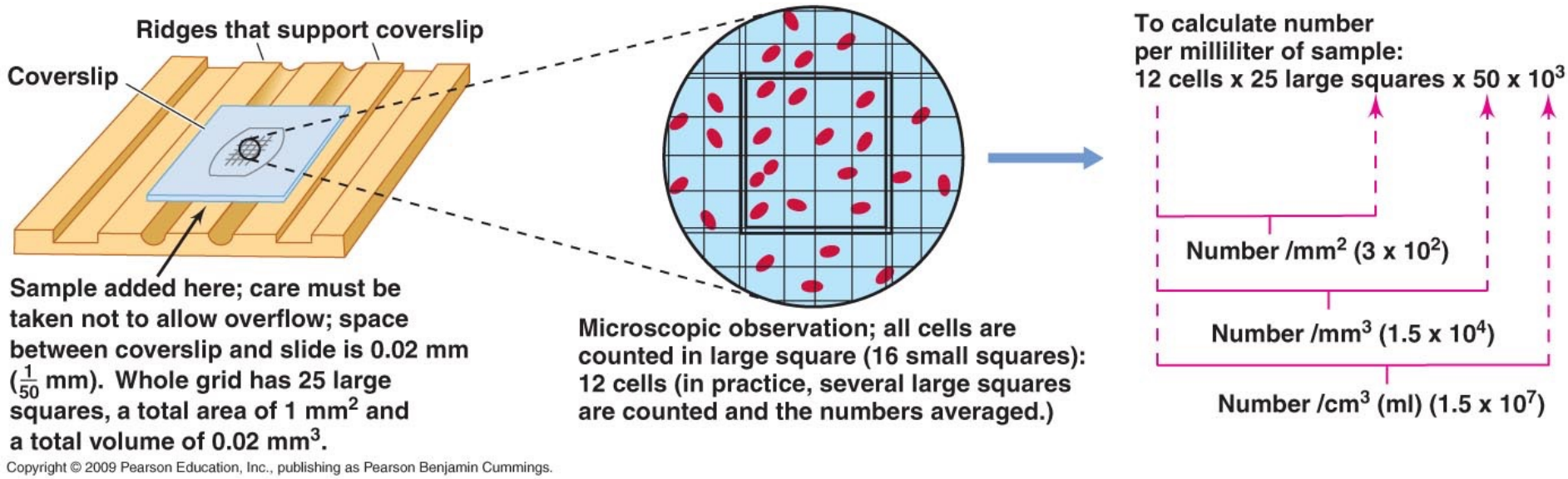
MEASURING MICROBIAL GROWTH:

1) **Total cell count**: using microscopy, cell counter.

2) **Viable count**: Also called **Plate** or **Colony count**.

3) **Turbidity measurements**: indirect measurement (spectrophotomer). Measures unscattered light. (called OD units when using a spectrophotometer).

Direct counts



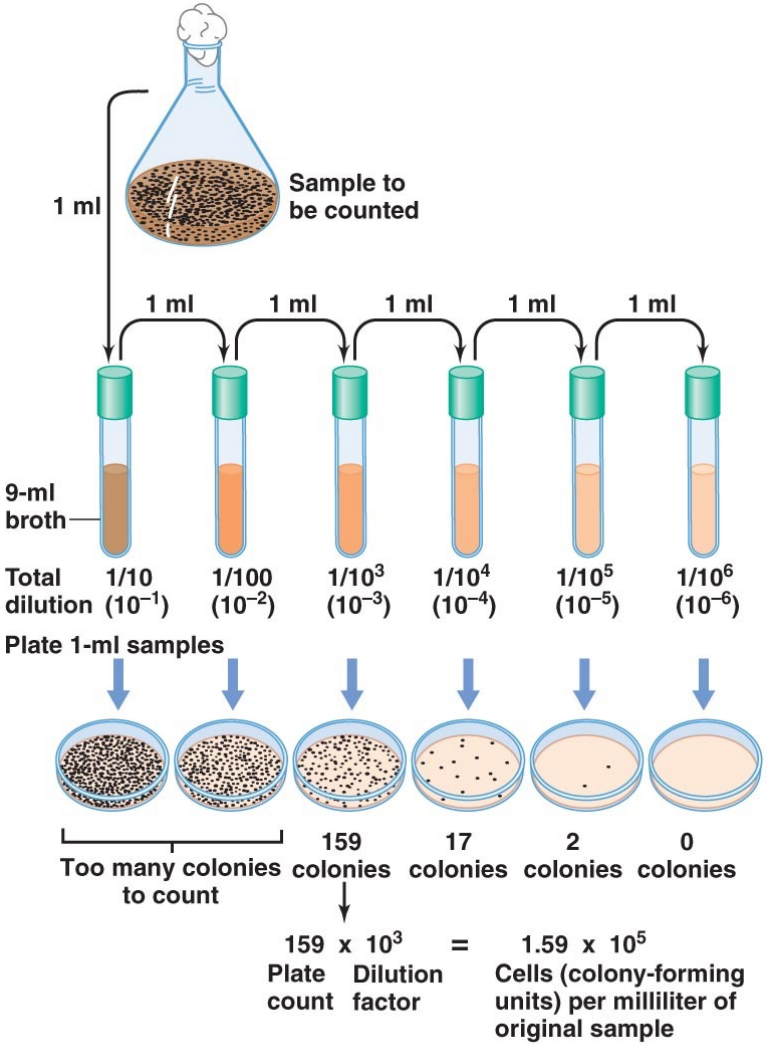
<u>Advantages</u>

- Quick and easy
- Get direct counts

<u>Disadvantages</u>

- Requires staining to distinguish live/dead
- Motile organisms must be immobilized
- Doesn't work well at low densities

Plate counts



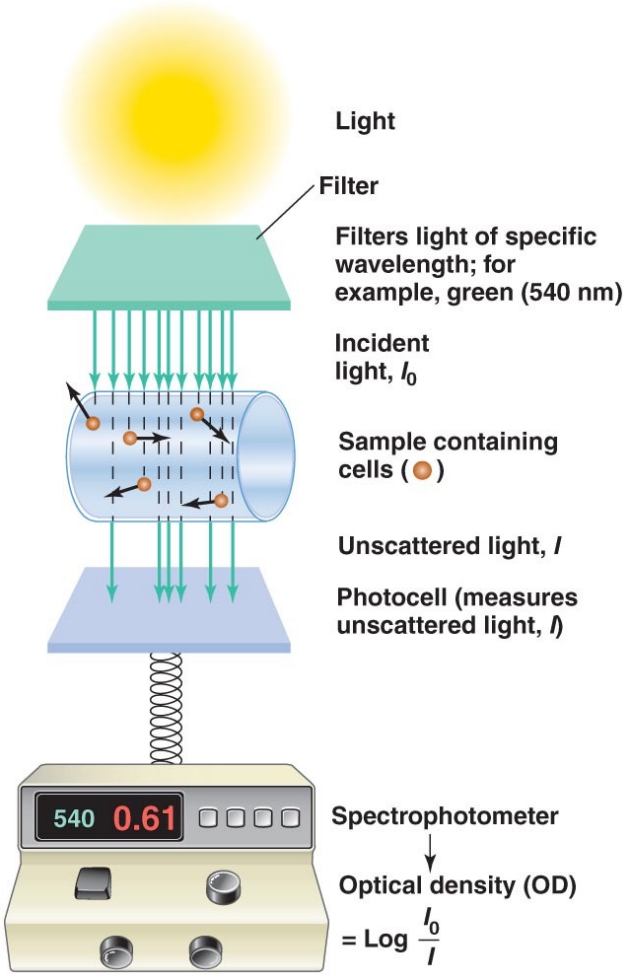
Advantages

- Can distinguish live/dead
- Reliable counts when growth conditions are known
- High sensitivity

Disadvantages

- Highly misleading for mixed cultures (the Great Plate Count Anomaly)
- Can take a long time for slower-growers

Spectrophotometer



Advantages

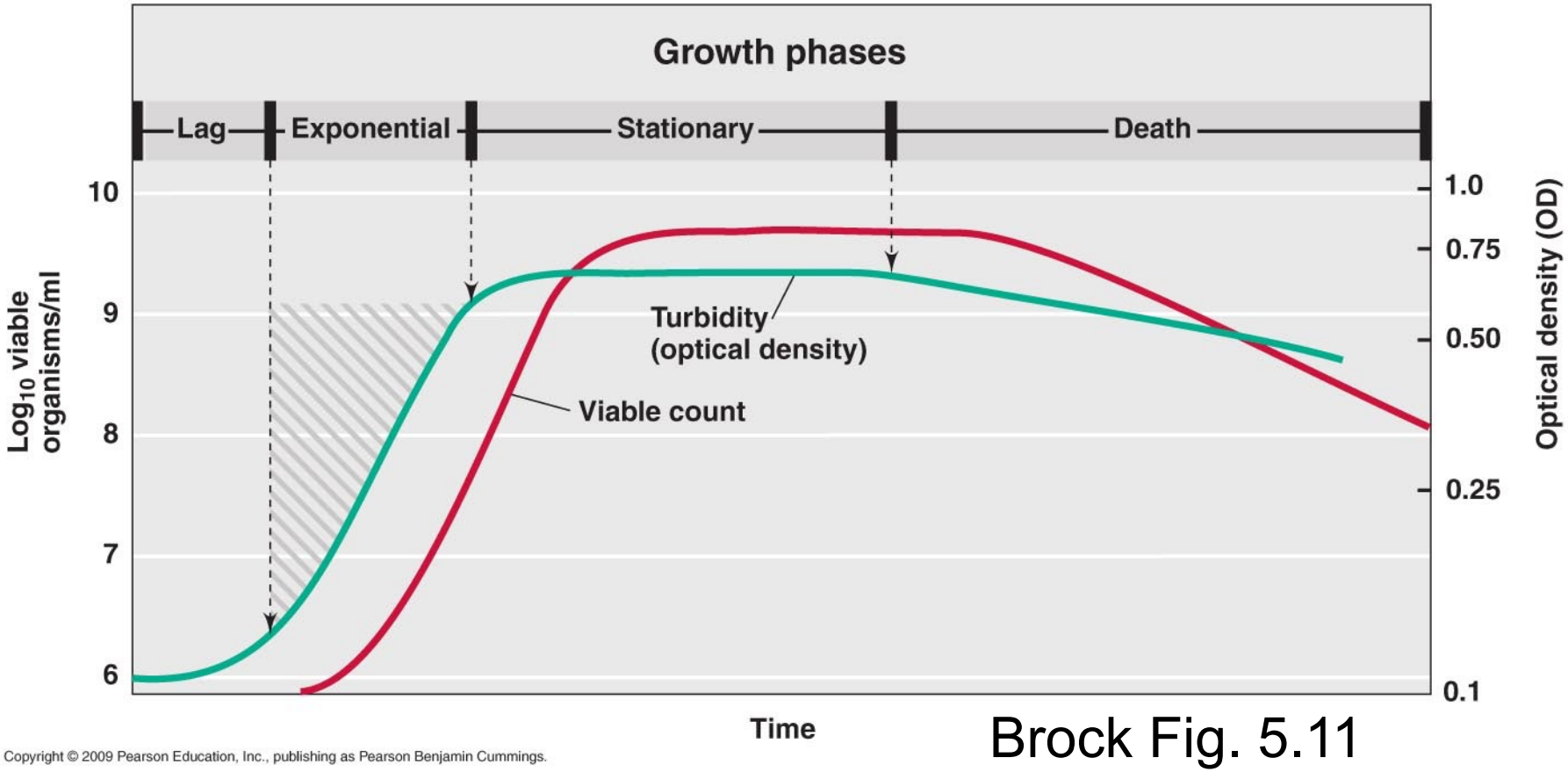
- Sensitive
- Can be done on live cultures
- Quick and easy
- Proportional to direct counts
- Can use the instrument for quantifying other samples (e.g. DNA, protein, enzyme activity)

Disadvantages

- Does not distinguish live/dead unless there is lysis.
- Misleading if clumps or flocs

THE GROWTH CURVE:

Growth cycle includes four phases.



Brock Fig. 5.11

Lag phase

- When microbial population is inoculated into fresh media lag time depends on:

1) age of the culture and
2) nutricional conditions.

Exponential phase

- Portion of the growth curve with a logaritmic growth.

Stationary phase

- Caused by lackage of essential nutrient and/or overaccumulation of waste products. No net increase or decrease of cell number.

Death phase

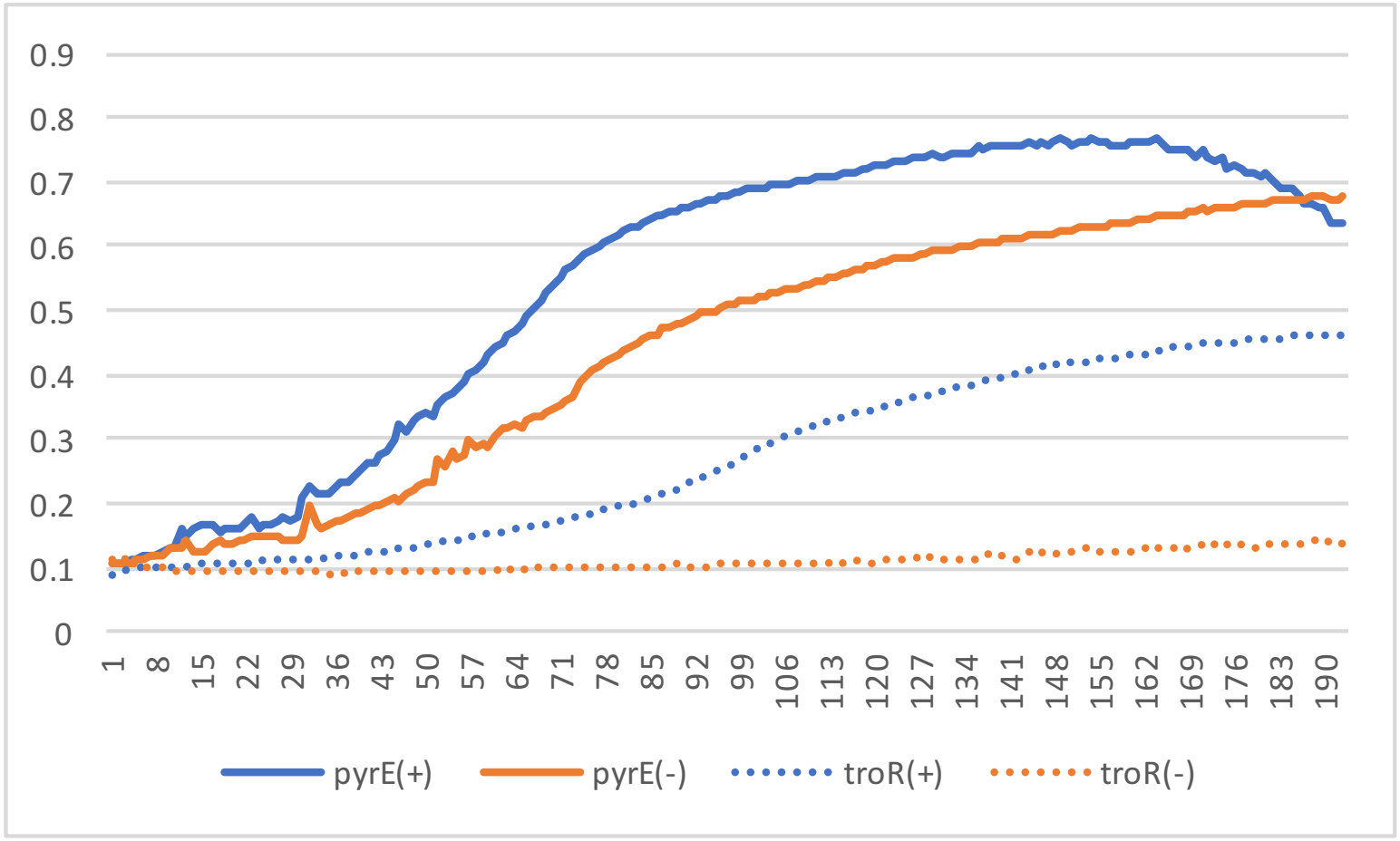
Introduction to Microbiology

Microbial growth

Introduction to the experimental design

Introduction to the experimental design

WHY DO WE CARE ABOUT GROWTH?



INTRODUCTION TO THE EXPERIMENTAL DESIGN

What is a mutant?

What is a KO mutant?

What do we use KO mutants for?

Media effect

MICROBIAL NUTRITION AND CULTURE MEDIA

Nutrient requirements for cell growth

Macronutrients are C, H, O, N, P, S, K, Mg, Ca, Na. Required for all organisms.

Micronutrients: Unnecessary to add to most routine lab cultures because already present. But must be added if using highly purified chemicals and distilled water.

Trace metals and vitamins, amino acids, purines, pyrimidines.

Culture media

A) Defined media (minimal media): highly purified inorganic or organic chemicals to highly purified distilled water.

B) Rich (complex) media: composition of the media includes “unespecific” compounds like digests/extracts of animal, fungal or plant products: beef, soybeans, yeast, malt or any highly nutritious substances

C) Selective media: Useful in clinical microbiology to detect presence or absence of a particular microorganism (example: Methylene blue dye inhibits growth of most gram + bacteria).

D) Differential media: media are used to differentiate closely related organisms or groups of organisms. Owing to the presence of certain dyes or chemicals in themedia, the organisms will produce characteristic changes or growth patterns that

are used for identification or differentiation.

How to determine the rate of growth of a cell population?

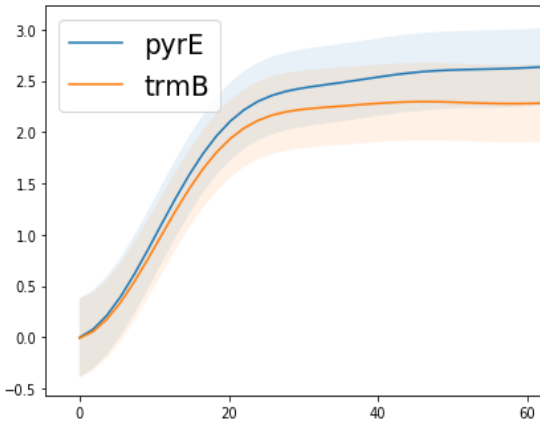
doubling time (which refers to the **generation time**)

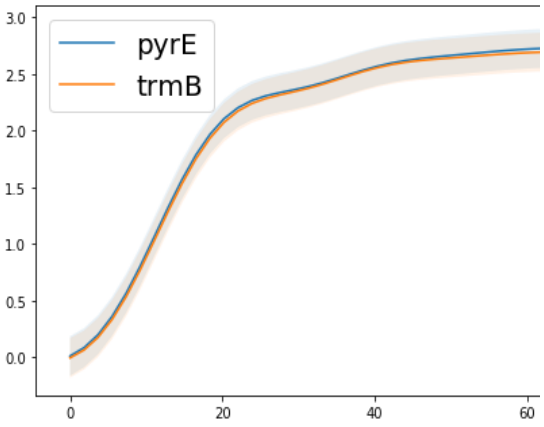
E.g., *E. coli* doubling time is 20-30 min.
Mycobacterium tuberculosis is 1-2 days.

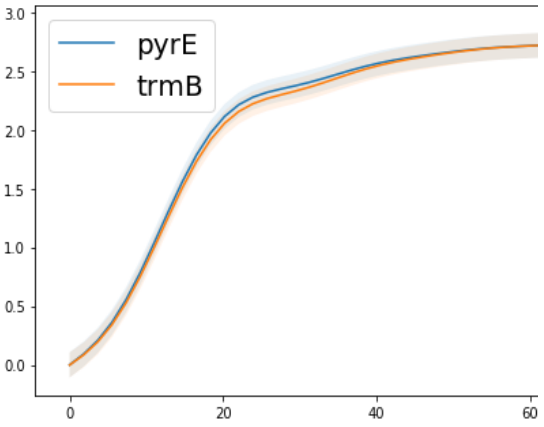
HIGH FREQUENCY GROWTH CURVE USING ROBOT

- Accetps two 100-well plates, up to 200 samples per experiment
- Constant temperature
- Shaking
- Measures OD every 30 minutes
- Measures multiple locations per well

EXPERIMENT HYPOTHESIS







	0.05% glu	0.1% glu	2% glu
Bayes:	178.3	9.74	22.3