7.02: Genetics Recitation One

September 12, 2001

Agenda:

I. Goals of Genetics Module
II. Aim of Today's Experiments
III. Growth of Cells in Culture
IV. Measuring Bacterial Growth
- **A. Optical Density**
- **B. Viable Cell Count**

V. Review of Sterile Technique
VI. Indicator Plates
VII. Bacterial nomenclature

I. Goals of Genetics Module:

A. Your scientific development
B. Generation and stabilization of *ara::lacZ* translational fusion

II. Aim of Today's Experiments: Learning more about *E. coli*, Done by

A. Following growth curve
B. Characterizing Phenotypes of different strains

Genotype: Genetic makeup of an organism
Phenotype: Characteristic way an organism interacts with its environment, visualization of genotype

III. Growth of Cells in Culture

A. *E. coli*'s normal habitat – human colon

B. Ways *E. coli* adapt to succeed in natural environment

1. Grow with other bacteria
2. Survive when nutrients are scarce
3. Use different nutrient sources
4. Low O_2

C. How growth in culture differs

1. No competitors
2. Abundant Nutrients
3. Constant Aeration

D. Growth Curve

1. **Characteristic pattern of growth demonstrated by cells grown in culture**
   - **Lag phase**: Adjusting to conditions
   - **Exponential (Log) Phase:** Rapid Growth, Maximal Doubling Rate
   - **Stationary Phase:** Growth rate slows, build up of toxins

E. Nutrient availability in media affects growth rate

1. Rich Media

- Abundant supply of organic materials and building blocks
- Poorly defined

2. Minimal Media

- Precisely defined
- Only contains components essential for growth

F. What you will be doing today

1. Grow E. coli strain BW140 at 37° C

a. BW140 is similar to the strain we will be using for Transposon mutagenesis

b. 37° C = body temperature

2. 1/2 of the class will grow their cultures in LB, the other 1/2 in Minimal media

a. Whose cells will grow faster? Why? Cells in LB will grow faster because they don't need to devote as much energy to synthesis of building blocks (amino acids, proteins, DNA & RNA bases) which are present in media.

IV. Measuring Bacterial Growth

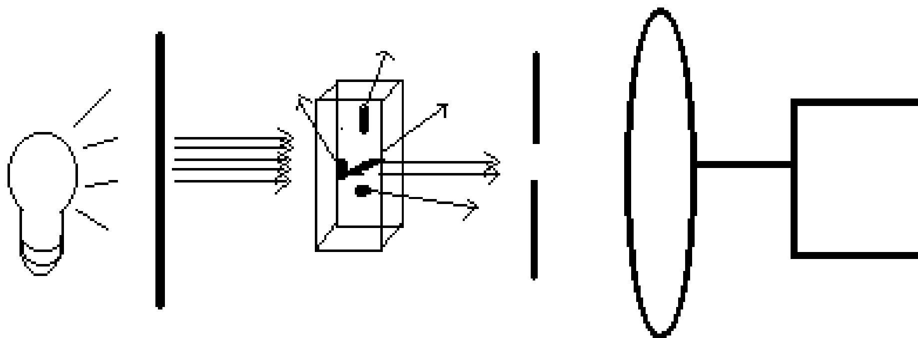
A. Optical Density Measurement

1. Advantage Unobtrusive way to measure growth

2. Light Scattering

A. Particles in your sample scatter light, even your media

B. Amount of light scattering proportional to amount of material present



3. **Non-linearity** When density of culture is too high, diffraction of light becomes abnormal. Therefore OD readings above 1 are considered inaccurate and it's necessary to dilute your sample in media before taking your reading.

4. **Disadvantage:** Measures dead cells

5. **Today's experiment**
    a. **Remove 1 ml sample – take OD_{550} readings with 0.9 mls**

    b. **Organize OD readings into chart.**

B. Viable cell counts

1. **Measure Number of live cells in your sample**
    Colony arises from a single cell

2. **Why do we use serial dilutions?**
    a. **Systematic way of reducing the amount of bacterial cells in your sample**

    b. **Today's procedure**

    c. **Would it be possible to dilute your cultures 10^{-6} in a single step?**
        It would not be very accurate, would require 0.1 ml in 100 L

V. Review of sterile technique

A. Goal : minimize contact with contaminants

B. Sterilize everything that contacts your bacteria

C. Ways to avoid contamination

1. **Transferring liquids**
    a. **Remove tube's cap, hold cap facing down**
    b. **Pass lip of tube through flame before and after removing liquid**
    c. **Flame lip of recipient tube before and after depositing liquid**
    d. **Place caps back on**

2. **Pipetting**
    Once you touch your pipet, it's no longer sterile. Avoid touching your pipet anywhere that comes in contact with bacteria

3. **Pipetman**
   Same is true for pipetman
   Closing tip boxes also reduces contamination

4. **Streaking for single colonies**
   Place lids face down to avoid allowing bacteria to settle in lid

If in doubt, get a new pipette, pipetman tip, or sterile pick!

VI. Indicator Plates

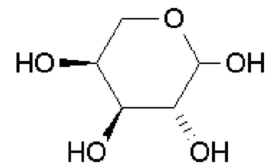
A. **Reagents present in media allow characterization of bacterial phenotypes**

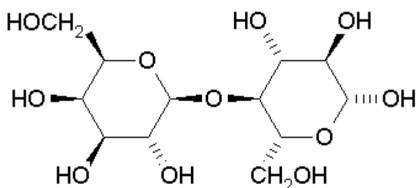
B. **Screening vs. Selecting**
   Screen: See all cells, cells of interest can be phenotypically distinguished
   Selection: Only what you're interested in grows

C. **Indicators we will be using today**
   1. **MacConkey** (Abbreviated Mac)
      a. **Contains a pH sensitive dye:**
         ↓ pH = red , ↑ pH = white/translucent
      b. **Sugar fermentation by bacteria causes pH ↓**
         Produces red colonies
      c. **Bacteria that can't ferment sugars use amino acids as a carbon source, producing ammonia.**
         pH↓, colonies appear white/translucent
      d. **Anaerobic respiration**

      e. **Two carbon sources:**

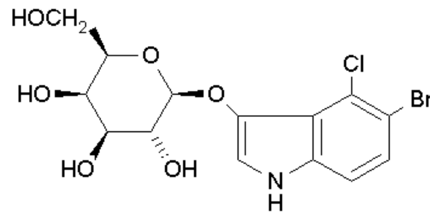
Arabinose (Ara)





Lactose (Lac)

2. **X-gal: 5-bromo-4-chloro-3-indolyl-β-D galactose**



a. **Substrate for β-galactosidase**
   - Enzyme encoded by *lacZ* – normal substrate is the bond between glucose and galactose in lactose
   - β-galactosidase cleaves bond between X group and galactose, liberating the X group, which is a blue color

b. **Inducible vs. Constitutive**

   Inducible = regulated by an inducer

   Constitutive = always on

Discuss again later in module, Professor Amon will also address in lecture.

3. **Kanamycin**
   a. **Antibiotic:** Inhibits Protein Synthesis
   b. **Select for cells that carry Kanamycin resistance**

4. **Phenotypes of the four strains you will be characterizing**
   a. **BK1: Ara-, LacZ+-inducible, Kan^R**
   b. **EJ1: Ara-, Lac-, Kan^R**
   c. **H24: Ara-, LacZ+-constitutive, Kan^R**
   d. **JET2: Ara+, Lac-, Kan^R**

	JET2
Mac Ara Kan	Ara + = red
Mac Lac Kan	Lac - = white/translucent
LB Kan	Growth
LB Ara X-gal Kan	LacZ- = white/beige

VII. Bacterial nomenclature

- **A. Communication of strain's genotype and phenotype**
- **B. Genes designated by 3 letter italicized name**
  1. **Wild type alleles -** Name followed by + sign

     lac+
  2. **Further subdivision of genetic loci -** Name followed by capital letter

     lacZ
  3. **Sites of mutation -** Name followed by number

     lacZ7
  4. **Deletions -** Denoted by Δ

     Δ(*lac)*U169
  5. **Double cross-over insertions –** Denoted by ::

     ara::lacZ
  6. **Alleles listed only if they differ from wild type** (Can't list all *E. coli* genes in genome – 4000 genes)
- **C. Phenotype designated by written description or abbreviation**