Outline for Today’s Lecture

• Overview of the week’s lab exercises
• For your assignments
  – Plotting growth curve numbers
  – Pubmed searches
  – Water Quality (due next Weds)
• Lecture about food contamination
• Group project proposals are due today
  – Feedback given back Weds; Revisions due next Monday 10/24

This week:

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This week

• Monday:
  – Bring in food sample and perform food lab (ex. 8)
  – Continue Water Exercise: do day 3 stuff
    • Gram stain of EMB positive colonies
    • Inoculate an EMB plate colony (positive) into 1X Lactose broth

• Weds:
  – Food lab, period 2:
    • Count plates--if colonies are too small, you can put back in incubator for longer and count later in the week, e.g. Thursday or Friday
    • Collect six single colonies/group. Restreak them for single colonies.
  – Water lab, day 4:
    • Examine Lactose broth tube from Monday

Assignment-Growth Curve-Overview

Due Weds

1. You need to present your results in a way that shows what you found. You can use Tables, Graphs (semi log or linear, which ever you find to be better) or Generation times
   • You will need to do at least two of the above to get full credit

2. Written description of your results, using good grammar and no spelling mistakes. I strongly advise you to use a word processing program to do this exercise. You will lose points for writing that is hard to read, or has spelling or grammatical mistakes.

3. Perform a Pubmed search for your antibacterial, and then do a second one comprised of your antibacterial+a bacterium you are interested in. Select one paper, and summarize the findings in one of the figures or tables. Include your search terms.
How to get doubling time from graph

• Culture must be in exponential growth phase to only take the points from that part
• Plot the log of the cell densities time
• Slope will give the generation or doubling time
• Can determine by just examining graph

Using Pubmed

• Google the term Pubmed
• Use it on campus for full journal access
• Search terms (1): ampicillin or streptomycin
• Search terms (2): streptomycin listeria monocytogenes
Water Quality Exercise is due Weds 10/26

1. There are two specific tables to prepare:
   1. MPN Analysis results
   2. Membrane filtration analysis results
2. Paragraph discussing why M-tec agar allows enumeration of E. coli
   - Again, please hand in something that is well written, clear, no spelling or grammar mistakes
   - Numbering is off in assignment, accidently numbered as “4”
3. Extra credit, do 2 above for mFC agar
Today we will analyze food for microbes

- Each group of four needs 40 grams of food
- Can drop it off (label the bag) in the classroom fridge

Foodborne disease

- There are many microbes associated with food
- Most are non-pathogenic
- Some are pathogenic, however.
- Fall into two main categories
  - Those that actually infect you--the bacteria multiply in your body
    - Pathogenic *E. coli* such as O157:H7, *Campylobacter, Salmonella, Listeria*
    - Usually proper cooking will kill these agents
  - Those that multiply in improperly stored food. Some may produce toxins that cause the illness.
    - In these cases, you do not get sick from the microbe but instead from its products
    - *Staphylococcus aureus, Bacillus cereus, Clostridia botulinum*
    - Cooking will oftentimes not inactivate the toxin so you will still get sick
Many of these agents are covered by FDA testing requirements and tracked by the CDC.

CDC Keeps track of numbers of cases of food-borne infection.
Salmonella

- Infection with bacterium (not toxin)
  - Gram -
  - Facultative Aerobe
  - Closely related to E. coli but cannot ferment lactose
  - Many different strains/serovars
- Diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment.
- Some people get very severe diarrhea and are hospitalized. In these patients, the Salmonella infection may spread from the intestines to the blood stream and can cause death unless the person is treated promptly with antibiotics.
- The elderly, infants, and those with impaired immune systems are more likely to have a severe illness.

Image: http://textbookofbacteriology.net/themicrobialworld/S.typhi.Fla.jpg
Salmonella epidemiology

• More cases in summer
• Kids are very susceptible
• Comes from animal feces--poultry, beef, reptiles--big ones are chicks, chickens, turtles, lizards, snakes
• Thorough cooking kills Salmonella.
• Wash your hands are handling birds or reptiles, or things that they touch!

Image: http://textbookofbacteriology.net/S.typhi.Gram.jpeg

Recent Salmonella Outbreaks
Source: CDC

Salmonella Outbreaks
2011
• Ground Turkey - Salmonella Heidelberg
• Whole, Fresh Imported Papayas - Salmonella Agona
• African Dwarf Frogs - Salmonella Typhimurium
• Alfalfa and Spicy Sprouts - Salmonella Enteritidis
• Chicks and Ducklings - Salmonella Altona and Salmonella Johannesburg
• Clinical and Teaching Microbiology Laboratories - Salmonella Typhimurium
• Turkey Burgers - Salmonella Hadar
• Cantaloupe - Salmonella Panama

2010
• Alfalfa Sprouts - Salmonella I 4,[5],12:i:-
• Shell Eggs - Salmonella Enteritidis
• Cheezy Chicken Rice Frozen Entree - Salmonella Chaster
• Frozen Mamey Fruit Pulp - Salmonella Typhi (Typhoid Fever)
• Restaurant Chain A - Salmonella Hartford and Salmonella Baidon
• Frozen Rodents - Salmonella I 4,[5],12:i:-
• Alfalfa Sprouts - Salmonella Newport
• Red and Black Pepper/Italian-Style Meats - Salmonella Montevideo
• Water Frogs - Salmonella Typhimurium
Clinical/Teaching Lab
Outbreak of 2011

• These people all had been in teaching labs or had contact with a person in a teaching lab
• In one case, they matched the outbreak strain to a commercially available Salmonella Typhimurium strain used in laboratory settings.

Advice to Students and Employees in Clinical and Teaching Microbiology Laboratories

Be aware that bacteria used in microbiology laboratories can make you or others who live in your household sick, especially young children, even if they have never visited the laboratory. It is possible for bacteria to be brought into the home through contaminated lab coats, pens, notebooks and other items that are used in the microbiology laboratory.

Persons working with infectious agents, including Salmonella bacteria, must be aware of potential hazards, and must be trained and proficient in biosafety practices and techniques required for handling such agents safely, including

• Wash hands frequently while working in and immediately after leaving the microbiology laboratory and follow proper hand washing practices. This is especially important to do before preparing food or baby bottles, before eating and before contact with young children.
• Do not bring food, drinks or personal items like car keys, cell phones and mp3 players into the laboratory. These items may become contaminated if you touch them while working or if you place them on work surfaces.

Do not bring pens, notebooks, and other items used inside of the microbiology laboratory into your home--or handle them carefully and remember that they were in the lab!

Wear a lab coat or other protective uniform over personal clothing when working in a microbiology laboratory; leave it in the laboratory when you are finished.

If you work with Salmonella bacteria in a microbiology laboratory, watch for symptoms of Salmonella infection, such as diarrhea, fever and abdominal cramps. Call your health care provider if you or a family member has any of these symptoms.
**E. coli** O157:H7

- Genetically different from lab *E. coli* but also gram-negative, motile, rod
- Has extra set of genes
- Got its name from a typing scheme
  - 1) In this scheme, researchers use antibodies to highly variable surface antigens. They have a bank of these antibodies, and basically just say that a given strain reacts with, for example, Antibody #26.
  - 2) Two antigens are tested
    - a) one is the O Antigen, aka LPS
    - b) one is the H Antigen, aka Flagellin
      » *O157* means it has O-antigen #157
      » *H7*: means it has flagellum #7
- Also called EHEC, for Enterohemoragic *E. coli*

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**EHEC Disease 1.**

1. You ingest bacteria in contaminated food
   - Has a very low infectious dose of ~ 10
2. Bacteria gets to your intestine and multiply.
   - You get abdominal cramping, nausea, bloody diarrhea
   - Other *E. coli*s cause diarrhea, but usually it is not bloody
3. Bacteria Adhere tightly to your cells
   - The type of adherence looks similar to another *E. coli*, called EPEC which is better studied (these notes are based on EPEC)
   - Cup-like pedestal under the *E. coli*. Attaching and Effacing.
   - Adhering bacteria can deliver proteins directly to the host cell
EHEC Disease 2

4. EHEC Produces a Toxin called VeroToxin
   - Being able to produce this toxin is the main difference between EPEC and EHEC
   - Highly related to Shiga Toxin (STX) from Shigella
   - Protein Toxin that is made by E. coli in the intestine but gets into the blood, and can travel to the kidneys
   - Cleaves host cell ribosomal RNA and stops protein synthesis
   - Our cells have receptors for Verotoxin, but only intestinal and kidney cells
     Kidney disease is called Hemolytic Uremia Syndrome
   - gene for verotoxin is carried by a temperate bacteriophage integrated into the genome.
     Can be passed readily from strain-strain; EHEC probably was created when an EPEC acquired the phage from a Shigella

EHEC is a commensal of cattle

- Resides in region of intestine called recto-anal junction
- Is shed in feces, and also ends up on hair
- Current strategy is to remove coat and clean the carcass in a separate area from subsequent processing
- Cook meat well

Several Recent EHEC outbreaks
(Source: CDC)

### Current big outbreak: *Listeria monocytogenes*

- Bacterium that grows inside you
  - Gm+
  - Can grow at 4°C
- Causing on-going outbreak associated with cantaloupes from one farm--have been recalled
  - Jensen Farms in Colorado
- Most infected people are over the age of 60; infection can also cause miscarriage
  - 116 infected, 23 deaths, 1 miscarriage
What we will do: Food Lab overview

• Each group of four is bringing in food item
  – You will need 40 g, which is about 40 ml of a wet food, or ~ 1.5 ounce

• Try to minimize hand exposure so that your veggie/fruit is not covered with hand bacteria…

Food Lab-safety

• Blender food up in the biosafety cabinet
  – Will have sign up sheet to make it all go smoothly
  – After blending, wait a few minutes before you open up the blender to let aerosols sink

• We will plate the blended food in two ways:
  – Mix with molten nutrient agar to disperse the bacteria throughout the agar
  – Streak some of the food onto EMB agar (same as used for water lab) to look for E. coli
Microbiological analysis of food

- Goal is to calculate the CFU/g

![Diagram showing the process of microbiological analysis of food](image)

Food Lab-data analysis

Lab Report Due 10/31

The "3" plate has:
- 10 CFU/0.1 ml
- 100 CFU/ml

The entire bottle has 200 ml

Bottle 2 was made from Bottle 1 by a 1:100 dilution

2x10^6 CFU/Bottle2 * 100 = 2x10^8 CFU/Bottle1

20 g of food/bottle 1

2x10^6 CFU/bottle1/20gm food = 1x10^5 CFU/gm
Group projects--

• **Today:**
  • People in your groups
  • Question you are asking
  • Hypothesis
  • Methods. This portion must be detailed enough so that we can evaluate whether we can do this experiment. So go to the literature or laboratory references. It must include media, materials etc.

• **Specific feedback handed back Weds; revision due next monday**

• **Revision should include a complete materials list for everything:**
  – Amount of media, recipes
  – Other things like swabs
  – Equipment like shakers, blenders, specs