20.106J – Systems Microbiology
Lecture 4
Prof. Schauer

- Reading for today: Chapter 6 – On Growth
- Problem set due today
- Today: Growth – in microorganisms it's different from in metazoans – increase in number of organisms instead of size
    - Binary fission
    - Other methods:
        - Organisms that replicate their DNA many times over, than split into many parts at once
- Next week: metabolic regulation

- Binary Fission
    - Time from bacterium to bacteria is a generation
    - Generation time is how long it takes
    - 20 minutes is a rather fast generation time. 8 minutes is the world record.
    - We look for bacteria that can replicate fast, or that can replicate in extreme conditions.
    - Cell content replicates before division.

- Fts proteins and the "divisome"
    - FtsZ aligns before division
    - The most intense signal occurs at the center edges due to the 3-dimensional shape

- Peptidoglycan synthesis
    - Peptidoglycan needs to be extgended for the cell to grow
    - The balance needs to be right, so cell integrity isn't compromised
    - Antibiotics bind to DNA binding proteins like FtsI, so that those enzymes aren't available for the peptidoglycan synthesis, and the bacterium lyses. (Autolysins without autolysis)
    - The FtsZ ring leaves a scar in the cell wall, which you can see later

- Peptidoglycan structure
    - Two planes with cross-links in between. These cross-links give it its integrity
    - MreB allows a variety of shapes -- not just spheres

- Exponential Growth
    - Because bacteria undergo binary fission, they can replicate into mind-boggling numbers very fast (exponential rate)
    - After two days of unregulated growth one bacterium's offspring would weigh more than the earth (assuming a 20 minute generation time)
    - Make a logarithmic plot of change in numbers as a function of time

- Growth Parameters
  - Write out equations
  - There will be homework problems relating to this growth
  - Related growth parameters

- The growth cycle
  - Why aren't bacteria always doubling? What limits their growth?
    - They exhaust their nutrients, causing the growth curve to level off
    - Build up of toxic waste products
  - The cell has to replicate everything before it divides
    - Therefore if you move a cell from a bad medium to a good one, there's a lag before it begins to grow.
  - Stationary phase – in a batch culture, for the most part things stay the same.
  - Death – in bacteria, this is exponential, like growth (very important)
    - It's not clear what's going on here – people have speculated.

- Total cell count
  - Demonstration: Prof. Schauer shows the class a counting chamber
    - Grid etched on with a laser
    - Two raised ridges – glass coverslip fits directly over, allowing you to measure the space between the platform and the coverslip – count through a microscope
    - The same concept and method is used for bacterial, blood cells, environmental samples, etc.
  - Problems with this method:
    - Not very precise
    - Hard to see
    - Doesn't distinguish live cells from dead ones
    - Requires phase contrast microscope to count unstained cells
    - Dilute samples must be concentrated

- Viable count
  - This is the more common method – dilute sample many times over
  - Demonstration: Prof. Schauer displays samples of test tubes with successive dilutions – each test tube is progressively less cloudy.
  - Then you plate the resulting tubes and wait for colonies to appear
  - You want to count a plate with between 30 and 300 cells
    - Otherwise the error becomes too high
  - Demonstration: Prof. Schauer displays agar plates resulting from each successive dilution
  - This kind of evaluation is difficult for slow-growing bacteria – you have to leave the plate to grow for up to a month.
  - This method doesn't work for bacteria that can't make colonies

        - These bacteria might be viable, but clump (you can use detergents to try to fix this problem)
        - Some organisms don't separate, but come in chains
    - Plating methods
        - Sometimes putting the agar on top is useful, because it stops the bacteria from moving around

- Turbidity as an indirect measure
    - Light scattering off of organisms
    - Depends on morphology of organisms – larger organisms scatter more light
    - You can quantify organisms by measuring the light scattering
        - Photometers
        - This is advantageous because you can still keep using the sample

- Chemostat culture
    - Instrument called a chemostat – bioreactor of sorts – you grow bacteria in it
    - Open system
    - Number of bacteria and rate of growth are kept constant
    - It enables you to control both the bacterial concentration and the doubling time.

- Cardinal temperatures: extremophiles
    - Temperature as an environmental condition – controls rate and yield
    - For every organism, you can determine maximum, optimum, and minimum temperatures for growth
    - The optimum is always closer to the maximum than it is to the minimum
    - Classes of organisms
        - Some organisms can grow in up to 113^{o}C
        - Organisms can grow anywhere that there's water
    - Psychrophiles
        - It's very clear why organisms can't grow at very high temperatures: proteins denature, etc.
        - However, it's less clear why they can't grow in low temperatures: you lose hydrogen bonding, but that's about all that changes
        - True psychrophiles, that prefer very cold temperatures, are rare
        - Those organisms can't handle warmer temperatures – therefore they live only in areas where it's cold all year round: the North and South Poles, glaciers.
    - Hyperthermophiles
        - Most of these are archaea
        - Archaea probably originated at very high temperatures: thermal vents, magma
        - They grow in superheated, high pressure water, over 100^{o}C

        - They have positive supercoiling of DNA – everything else on earth has negative-coiled DNA
        - Problems with membrane stability – remember, archaea have different membranes from us (eukaryotes can never grow above $50^{o}C$

- Thermophiles
    - Important source of enzymes for biotechnology
        - Differently colored band at Yellowstone: each colored band is a different thermophile

- Extremophiles of pH and osmolarity
    - They maintain their internal cell environment
        - They don't, for example, have such low pH or such high salt concentration inside the cell as they do outside
    - Accumulate inorganic ions or make organic solutes
    - Compatible solutes
    - Note: freezing is similar to dehydration: what kills cells as they freeze is the loss of H_2O as it forms into crystals
    - Demonstration: Prof. Schauer shows the class a device for creating an anaerobic atmosphere for growth
    - Toxic forms of oxygen