

Bacterial Growth- Microbiology Test 2

I. Binary Fission

- A. start off with wall enlargement zone
 - 1. part of cell where cell division takes place
- B. if zoom in at higher power, see...
 - 1. wall band of outer membrane of cell wall
 - a. it's really a 3-D ridge that goes completely around outside of cell
- C. later...
 - 1. wall band splits to form wall notch between 2 bands
 - 2. inner membrane of cell wall invaginates
 - a. it's called the nascent septum
 - 3. cell membrane starts to invaginate as well
- D. cell notch widens as new wall is added near septum and inner mem of cell wall invaginates more
 - 1. insertion of NAG's and NAM's- peptidoglycan
 - 2. in spherical cells, new wall added at equatorial position- near septum
 - 3. in rod cells, new wall is added at lateral positions as well (not near septum)
- E. DNA replication is occurring at the same time
- F. cell wall forms 2 cells, each with wall band ready to start process again
- G. s/t have cell division without cell separation:
 - 1. dividing in 1 plane w/o separation forms a chain of spherical cells=streptococcus
 - 2. dividing in multiple planes w/o sep forms a cluster of cells=staphylococcus

II. Generation Time

- A. $T=I+C+D$
 - 1. T =generation time/doubling time, time it takes for 1 cell to become 2
 - 2. I =time to produce initiator
 - a. initiator is a protein that has to be made in specific amounts to start replication
 - 3. C =replication of DNA
 - 4. D =time between the end of replication and cell division (for slow-growing cells)
 - a. have 2 copies of DNA, then divide cell w/ process of wall bands and notches
- B. generation times depend upon the type of cell:
 - 1. G- rods have gen time of 20-30 mins
 - 2. G+ cocci have gen time of 45-60 mins
 - 3. acid-fast cells have gen time of up to 24 hours

III. Replication For Slow-Growing Cells

- A. have double-stranded DNA with strands all held together by H-bonding
- B. unwind one area of DNA so have single-stranded region=replication fork
- C. make a copy of each DNA strand by using DNA polymerase to add the complement bases
- D. as you unwind more, the replication fork moves
- E. at the end, have 2 double-stranded DNA's
 - 1. semi-conservative replication- $\frac{1}{2}$ DNA remains the same- 1 old strand, 1 new strand

IV. Replication For Rapidly-Growing Cells

- A. rapid growth→lots of protein synthesis→lots of initiator synthesis→multiple replication forks
- B. the same thing as slow-growing, except start new replication forks while still using old one
- C. leads to more copies of DNA
- D. significance of multiple replication forks has to do with gene dosage
 - 1. gene dosage=number of copies of a particular gene
 - 2. more copies of a gene near original replication fork
 - 3. can have change in physiology of cell based on number of copies of a gene

V. Measuring Growth

A. ways to measure cells/mL (1 of 4 ways)

1. packed cell volume
 - a. involves use of centrifuge
 1. device used to settle cells (pellet) to bottom of tube, so clear liquid is above it
 2. can pour off liquid and measure growth of cells
 3. does not differentiate b/t viable and non-viable cells
2. spectrophotometry
 - a. use a spectrophotometer to measure how much light passes through a culture
 1. measures difference b/t light coming in and light coming out
 2. don't use wavelength that medium will absorb
 - b. measurement=optical density
 - c. need to first set up standard curve of optical density vs. cells/mL
 - d. once get optical density, look at curve and see how many cells/mL in that flask
 - e. does not differentiate b/t viable and non-viable cells
3. measure cell component
 - a. measure ATP, protein, or DNA b/c easy to measure
 - b. use standard curve of mg protein vs. cells/mL
 - c. measure mg protein in flask and refer to standard curve to get cells/mL
 - d. does not differentiate b/t viable and non-viable cells
4. microscopy
 - a. uses flat slide with depression
 - b. when put on coverslip, have known volume underneath it
 - c. have checkerboard of boxes on slide and can see it when look through microscope
 - d. can count number of cells in box and then know cells/mL b/c know volume
 - e. does not differentiate b/t viable and non-viable cells
5. viable counts
 - a. have flask with bac culture and want to know cells/mL
 - b. set up series of dilution tubes, each with 9 mL of broth
 - c. put 1 mL from flask into 1 of the tubes so have 10^{-1} dilution
 - d. put 1 mL of that into next tube, so have 10^{-2} dilution
 - e. keep doing this=serial dilution
 - f. if have 10^{-6} dilution in last tube and after incubation of 1 mL on petri dish, it has 100 colonies, then...
 1. originally it had $10^2 \times 10^6 = 10^8$ cells/mL
 - g. if plate same dilution w/ .1 mL and get 100 colonies, then ...
 2. originally it had 10^9 cells/mL b/c have to multiply by 10 b/c it's .1mL
 - h. this is the only way that gives a viable measurement

B. to calculate number of cells at the end of growth, use formula: $B = B_0 \times 2^n$

1. B is the number of cells at the end of a period of growth
2. B_0 is the number of cells at the start of that period of growth
3. n is the number of generations/number of doublings
4. if start with a single cell...
 - a. after 2 generations, B=4
 - b. after 3 generations, B=8

5. if start w/ 1 million cells...
 - a. after 2 generations, B=4 million
 - b. after 3 generations, B= 8 million
6. if there were no limits to growth, if have single cell w/ n= 20 mins, 48 hrs later...
 - a. this is 144 generations and would have $1 \times 2^{144} = 2 \times 10^{43}$ cells.
 - b. if each individual cell weighs 10^{-12} g, would weigh 2×10^{31} g which is 4,000 times planet Earth's weight

VI. Bacterial Growth Curve

A. description of graph

1. plot the log of the number of viable cells vs. time
2. take a small sample of cells that grew up overnight and put them into fresh medium- time 0
3. first no change in number of cells=lager phase
4. then period of doubling/logarithmic growth=log phase
5. number of cells stays the same/levels off=stationary phase
6. number of cells starts to decrease=death phase

B. limits to growth of a cell

1. nutrient depletion
 - a. run out of C sources, amino acids...
2. buildup of toxic products, usually acids
 - a. as acids increase, pH decreases- becomes too acidic
 - b. could be other toxic molecules produced that could accumulate and inhibit growth

C. steps to growth

1. lag phase
 - a. cell has to...
 1. adapt to new medium=adjust its internal pH from acidic to around 7
 2. take up nutrients from medium before it can grow
 3. synthesize new enzymes
 - A. was process of protein degradation b/f, so need new copies of protein
 4. induce enzyme synthesis- go through regulatory processes to decide whether to make protein
 - A. s/t need to synthesize regulatory factors to turn on genes to synthesize enzymes
 - b. increase in cell size w/o an increase in cell number
 - c. length of lag phase depends on a number of factors
 1. length of time of previous stationary phase from when incubated culture
 - A. long stat phase (1-3 days)
 - 1.culture is depleted, missing nutrients and proteins, very acidic
 - 2.needs long lag phase
 - B. short stat phase (2-3 hours)
 1. culture has proteins and nutrients, not too acidic
 2. needs short lag phase

2. composition of medium
 - A. rich medium
 1. has amino acids, vitamins, and nutrients, so culture can get them easily
 2. needs short lag phase
 - B. minimal medium
 1. only has glucose, so needs to make vitamins and amino acids
 2. needs long lag phase
 3. nature of organism- vary in efficiency and mechanism
 - A. some org do things rapidly
 1. need short lag phase
 - B. some org do things slowly
 1. need long lag phase
2. transition phase
 - a. there's a smooth curve b/t lag and log, not sharp break b/c...
 1. not every cell is ready at the same time for log phase
 2. not every reaction in the cell is ready to proceed at its log phase rate
 3. log phase
 - a. growth is logarithmic- a curve
 1. therefore, plot log number of cells vs time, not number of cells vs time
 2. this gives a straight line, so can calculate generation time
 3. slope of line shows rate of growth
 - b. this is a period of...
 1. rapid growth
 2. rapid metabolism
 3. balanced growth
 - A. every 20 mins, cell wall, rib, and chro are doubling- e/t coordinated
 4. transition phase
 - a. smooth curve b/c
 1. not every cell gets depleted at the same time
 2. not every cell slows down at the same time
 5. stationary phase
 - a. 2 points of view about what's happening:
 1. growth stops, culture runs out of nutrients, becomes too toxic so levels off
 2. cell growth is exactly equal to cell death
 - b. this a period where...
 1. storage molecules are being made
 2. synthesis of capsule begins
 3. some cells make antibiotics- for 2 reasons:
 - A. limited food supply, so kill competition to gain more nutrition
 - B. to lyse the other bac cell to get nutrients from inside it
 4. sigma factors appear
 - A. stationary phase is a new state of existence, so need new factors
 - B. they turn off log genes and turn on stationary genes

6. death phase

- a. this is a period of depletion of energy reserves
- b. therefore, have loss of selective permeability
 1. b/c can't do active transport
- c. lysis=cell death b/c can't regulate ions into and out of the cell
- d. death is exponential like log phase
 1. lose a certain fraction of population per unit time
 - A. under light microscope these cells look different
 - B. cells are elongated, swollen, and distorted
- e. rate of death depends on:
 1. environment
 - A. how starved, toxic, acidic it is
 2. nature of organism
 - A. some fragile- start dying when not enough nutrients
 - B. some can withstand harsh conditions

D. from bac growth curve, get 2 parameters:

1. from slope of log phase- get rate of growth- calculate generation time/doubling time
2. yield
 - a. amount of new growth that you get- difference b/t lag phase and stationary phase
 1. low yield- slightly cloudy tube
 2. high yield- tube thick with cells
 - b. depends upon conc of ATP in the cells
 1. some org more efficient at producing ATP, so will get more cells
 2. some medium better for production of ATP
 - A. since biosynthesis requires ATP, if have molecules available in medium, cell will have more ATP b/c won't have to make them

E. limits to growth of a colony

1. nutrient depletion
 - a. oxygen has to diffuse through all cells to get to the bottom, stops after a while
2. toxic product buildup

F. kinds of info we get from growth curve

1. info about organism itself
 - a. some grow faster, others slower
2. info about medium
 - a. some support rapid rate of growth, others slower
3. add/vary C-source
 - a. see if it supports rapid rate of growth, slow growth, or no growth
4. add/vary molecule such as antibiotic
 - a. see if it will inhibit growth of these bac cells

G. chemostat

1. special machine
 - a. flask with bac that has spout just above level of bac
 - b. can constantly put in fresh medium and old can spill out
2. cell heaven
 - a. continuous log phase
 - b. no limits to growth
 1. never runs out of nutrients b/c keep adding
 2. no overcrowding b/c some spills out
 3. no toxic waste b/c replacing medium
3. can use these cells for a study, except by cell division need synchronous growth

H. ways to get synchronous growth

1. membrane elution (age)
 - a. pass culture through filter paper, so liquid goes through and cells stick to paper
 - b. turn paper upside down while cells stick to it and pass fresh medium through
 - c. cells divide while attached to filter paper and daughter cells fall off
 1. daughter cells are at the same stage of cell division
 - d. when use these cells for inoculation, get synchronized growth
 1. log phase of bac curve looks like staircase rather than straight line
2. filtration (size)
 - a. take a stack of filter papers and pass culture through
 - b. only smallest fit through=youngest
 - c. use these cells as inoculant for growth curve experiment- get synchronized growth
3. start off with one variable, stop it, and then start it again
 - a. temperature
 1. grow culture at 37 degrees, put briefly at 25, then transfer back to 37
 2. 2 assumptions:
 - A. enzyme is temperature-sensitive and only functions at 37, not 25
 - B. this enzyme is part of the process of cell division- critically needed
 3. at 37 degrees, cells not synchronized
 4. at 25 degrees, all steps not affected continue and others stop
 - A. e/t remains stopped at point needing that enzyme
 3. when restore back to 37 degrees, e/t is synchronized
 - b. thymine
 1. put thymine negative mutant in medium with thymine, transfer to medium w/o thymine, then put back into medium w/ thymine
 2. thymine negative mutant means gene is mutated so cell cannot make thymine
 - A. thymine is one of 4 bases of DNA
 - B. chromosome replication is 1 step in the process of cell division
 3. therefore, need to feed that cell thymine so it can grow
 4. bac grows in medium w/ thymine, but growth is not synchronized
 5. when transferred to medium w/o thymine, bac grows until step needing thy
 - A. every cell is stopped at the same point
 6. when put back into medium w/ thymine, growth is synchronized

VII. Bacteria In The Body

- A. drug-resistant pathogens in the gut
 1. very few of them, don't multiply a lot b/c of nutrient competition w/ normal flora
 2. but person w/ other serious problems, such as fever, takes antibiotics
 3. antibiotics sterilizes gut and destroys normal flora, so pathogen can grow and become many
 - a. ex: Clostridium difficile causes enterocolitis
 4. there's always a risk vs benefit calculation when prescribing antibiotics
- B. some antibiotics only kill growing cells
 1. ex: penicillin
 - a. inhibits cell wall synthesis by internal osmotic pressure, causing lysis
 - b. so if cell not growing or dividing, it doesn't have a target
 2. only kill log phase cells, not stationary phase cells
 - a. if have abscess=rotting tissue, not conducive to growth, these antibiotics won't work

VIII. Time Frame for Growth Curve

- A. growth curve for organism w/ generation time of 20 mins
 - 1. lag phase: 20-30 min
 - 2. log phase: 6-8 hrs
 - 3. stationary phase: 4-5 days
 - 4. death phase: 6-7 days
 - 5. total: around 10 days
- B. take antibiotics until the end of death phase
 - 1. this is b/c at any stage, there are still some cells left alive which can start a relapse
 - 2. different lengths of time to take antibiotics
 - a. G- cells: take for 7 days
 - b. G+ cells (slower growing): take for 10 days
 - c. acid-fast cells: take for 1 yr
 - 1. ex: those bac that cause tuberculosis
 - 2. biggest problem is patient compliance
 - A. monitor-nurse or healthcare worker-may remind patient to take meds
 - B. do this b/c tuberculosis is highly contagious

IX. Planktonic Growth vs. Biofilms

- A. planktonic growth
 - 1. free-living bac (in liquid culture)
 - 2. characterized by motility- have flagella synthesis
- B. biofilms
 - 1. the way bac exist in the real world
 - 2. flat, thick growth on a surface
 - a. has special features- towers, mushrooms, and channels
 - b. all of this is enclosed in an exopolysaccharide
- C. as planktonic converts to biofilm, free-living genes are turned off and biofilm genes are turned on.

X. Steps to Forming Biofilms

- A. attachment
 - 1. reversible attachment
 - a. cell bounces on and off surface
 - b. even at that pt, have surface-induced gene expression- contact causes change in cell
 - 2. irreversible attachment
 - a. flagella rotation is being turned off
 - b. even at this pt, have quorum sensing
 - 1. products are produced and having an effect b/c have a minimum number of bacteria present
 - 2. function of group, not an individual cell
- B. aggregation
 - 1. twitching motility turned on
 - a. bac on surface sense each other and use Type IV pili to walk across surface
 - 2. leads to microcolony formation (as bac move toward each other)
- C. biofilm formation
 - 1. characterized by exopolysaccharide synthesis
 - 2. horizontal spread of bacteria
 - 3. in some places, have vertical growth- piling up of cells
 - a. forms towers and mushrooms
 - b. some structures can be 100 microns thick
 - c. within structures, have channels in contact with surface
 - 1. medium is able to come in and circulate through them

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XI. How Cells in Biofilm Get Nutrients

A. diffusion through biofilm

1. doesn't work for thick areas of biofilm

B. channels

C. exopolysaccharide ligase

1. digests the polysaccharide (chain of sugars), so it can be used for nutrition

XII. Properties of Biofilms

A. gene expression

1. induce biofilm-specific gene expression, such as the exopolysaccharide
2. repress planktonic-specific gene expression, such as genes for flagella synthesis
3. microarray technology can be used to look at all genes of a cell
 - a. 800 proteins increase or decrease b/t planktonic growth and biofilms

B. cells are dormant- not growing

C. antibiotic resistant

1. takes 1000X more antibiotics to kill cells in biofilm compared to cells growing in solution
2. 4 reasons why:
 - a. thickness of biofilm
 1. cells encased and shielded from antibiotics
 2. but since antibiotics small like nutrients, doesn't really hold true
 - b. exopolysaccharide neutralizes antibiotics so they're not effective
 - c. antibiotic efflux pump
 1. as cells become biofilm, genes are induced to form this pump
 2. pumps antibiotics out of cells
 - d. cells are dormant and some antibiotics only work on growing cells

D. phagocyte resistant

1. WBC can't get rid of cells when they're "hidden" in a biofilm
2. antibodies, proteins, and a/t else that usually gets rid of bac can't when they're in a biofilm

E. intercellular DNA exchange

1. bac can exchange DNA from 1 cell to another even in solution
2. happens more frequently in biofilm state b/c have more cells, closer together, not moving

F. intercellular communication

1. refers to quorum sensing
2. signal molecules diffuse b/t 1 cell and another
 - a. in G- cells, main signal molecule is Acyl Homoserine Lactone
 - b. in G+ cells, main signal molecule is peptide
3. essential idea is concentration
 - a. cells can make these molecules individually, but n/t will happen b/c not enough
 - b. high conc affects gene expression in other cells
 - c. can also be autoinducers
 1. when signal molecules are absorbed by cells that made them, it causes cells to make more of the same molecules
 - d. quorum sensing is property of groups, not individuals
 - e. this has been used as model for development

XIII. Ways of Release of Cells from Biofilms

A. swarming

1. release of planktonic cells- cells are released and go back to planktonic growth
2. biofilm is a source of seeding of pathogens
3. bac cells hide out in biofilm and then can be released and cause disease

B. rolling

1. a chunk of biofilm breaks away from the mass and rolls away
2. this spreads the biofilm to other areas in the body- dangerous b/c resistant to antibiotics...
3. can form an embolus- a sizeable chunk of material that blocks flow of blood in bloodstream
 - a. can cause a heart attack or stroke

C. rippling

1. the whole biofilm moves somewhere else

XIX. Current Biofilm Concerns and Research

A. medical issues

1. biofilms can form on many surfaces:
 - a. teeth- forms dental plaque
 - b. catheters
 - c. tracheotomy tubes
 - d. heart valves
 - e. artificial joints- knees and hips
 - f. over and above thick mucus and lung tissue of cystic fibrosis patients
 1. pseudomonas is the organism that forms the biofilm
 2. causes chronic lung infections and other problems associated with biofilms

B. new areas of research

1. coat materials (e.g., joints, catheters) with antibiotics to prevent initial biofilm formation
2. use biofilm resistant material so bac don't stick
3. new targets for drugs- develop drugs that...
 - a. block quorum sensing so it's easier to get rid of bac
 - b. block biofilm formation

C. commercial issues

1. biofilm formation in boilers, drinking water pipes, hulls of ships

D. household issues

1. biofilms form on shower curtains
 - a. can be dangerous to someone with weakened immune system

E. new ideas

1. with regard to medicine
 - a. have new paradigm for infectious disease
 1. acute disease due to planktonic growth
 2. chronic disease (long-term) due to biofilms
2. with regard to biology
 - a. beginning to think of bac as multicellular
 1. talking about the whole biofilm as an organism
 2. there's division of labor
 - A. ex: some produce polysaccharide ligase to digest polys. while others use the product of this reaction and grow
 - b. biofilm consists of multiple species
 1. can use language of ecology- different org living together
 - A. cross-communication=interactions b/t species (e.g., quorum sensing)
 - B. specialization- dif species have dif roles in biofilm
3. Bill Costerton started the work on biofilms.