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Factors Affecting Bacterial Growth- Microbiology Test 3

I. Temperature
- A. w/ relation to growth rate
  - 1. minimum temp
    - a. farther to the left is too cold
  - 2. maximum temp
    - a farther to the right is too hot
    - b. temp affects protein structure, usually enzymes, which affects protein function
      - 1. protein can open up and fall apart, so won't function
      - 2. protein can squish together, no 3-D structure, so no function
    - c. as temp is increased
      - 1. protein affected is nonessential for growth, so growth rate not affected
      - 2. enzyme affected is essential for growth, but can supply a product (ex: amino acid product of a pathway), so growth rate not affected
      - 3. protein affected is essential for growth, but can't supply a product, so growth is inhibited, leading to maximum
  - 3. range
    - a. the temp at which bac can grow
  - 4. optimum temp
    - a. best temp for growth rate, not nec best temp for sporulation or pigment production
    - b. two things happening simultaneously
      - 1. activation is increasing
        - A. talking about collision theory- molecules moving faster, so collide more, so more activity, so enzyme works more
      - 2. inactivation is increasing
        - A. talking about the breakdown of the 3-D structure of the protein, losing shape and specificity, as temp increases
    - c. optimum temp is the greatest difference b/t activation and inactivation
    - d. as temp is increased, act (collision) predominates, but then there's a major effect on enzyme structure (inact), causing growth rate to decline. After max, protein structure is destroyed, so there's no growth.
- B. classification by temp
  - 1. psychrophiles
    - a. optimum is 5 degrees Celsius
    - b. ex: Clostridium botulinum vegetative cells (G+ rod, anaerobe, spore-forming)
      - 1. produce exotoxin that causes botulisim- form of food-poisoning
        - A. putting food w/ these cells in fridge helps bac cells grow
  - 2. mesophiles
    - a. grow at midrange temp: 10-50 degrees Celsius
      - 1. those in soil have optimum around 25-35 degrees Celsius
      - 2. those in body have optimum around 35-45 degrees Celsius
        - A. most bac in body grow around 37 degrees C
        - B. bac on skin have optimum around 32 degrees C
    - b. most pathogens have optimum of 37 degrees- body temp
  - 3. thermophiles
    - a. optimum around 45 degrees C
    - b. many prefer hotter temp- can grow in 80-100 degrees C

C. heat shock response
1. raising temp causes certain changes to take place due to appearance of new sigma factors
2. sigma factors bind to RNA polymerase and give specificity to transcription
3. heat shock genes are transcribed, so heat shock proteins are produced
a. HSP 70
1. prevents aggregation of newly synthesized proteins
2. stabilizes unfolded proteins
b. HSP 10 and HSP 60
1. molecular chaperones
2. catalyze the correct folding of misfolded proteins
c. proteases
1. remove denatured or misfolded proteins
2. do this by breaking down proteins into individual amino acids

D. factors that determine bacterial sensitivity to heat
1. temp
a. how hot it is makes a difference to how sensitive bac will be
b. to kill a population of C. botulinum spores...

Temp	Time To Die
100 C	6 hrs
105 C	2 hrs
110 C	30 mins
115 C	12 mins
120 C	5 mins

c. to kill thermophile vegetative cells

Temp	Time To Die
100 C	19 hrs
110 C	3 hrs
120 C	17 mins

2. number of organisms
a. physical
1. clumping of bac makes them more resistant to heat
2. this is b/c they have a shielding effect- cells outside protect
b. physiological
1. refers to cells in death phase
2. large numbers of enzymes are already non-functional
3. increasing temp has little effect b/c destroying dead enzymes
3. species
a. makes a dif whether the cells are psychrophiles, mesophiles, or thermophiles

4. medium
    a. composition
        1. lipids and proteins in the medium protect
        2. bac adhere to surface of micelles= drops of lipids or proteins
        3. this makes bac more resistant to heat b/c shielded on one side
    b. viscosity
        1. the more viscous (thick) the medium, the more it protects
    c. depth
        1. the farther cells are from the heat source, the more they're protected
5. pH
    a. to kill C. botulinum...

PH	Time To Die
7	6 hrs
5	1 hr
4	15 mins

    b. if pH is too acidic or too alkaline, enzyme is weakened
    c. increased heat weakens an already weakened enzyme, having a bigger effect
    d. for medical microbio, need to consider all 5 factors that make bac sensitive to heat

E. freezing bac
1. slow freezing
    a. kills 3 ways
        1. osmotic pressure increases b/c conc of solutes increases
        2. conc of toxic products increases
        3. ice crystals form inside cells and puncture membranes, disrupting them
            A. use this idea to break open cells in lab
            B. lab procedure=freeze-thaw technique
            C. freeze and thaw cells until ice crystals puncture membranes
            D. left with enzymes and ribosomes, so can use them
2. rapid freezing
    a. go down to -80 degrees Celsius in ½ -1 min
    b. results in preservation
    c. left with amorphous solid (no ice crystals)
    d. vacuum dry sample to remove water through sublimation- goes from solid to gas
    e. this freeze-dry technique is called lyophilization
    f. can hold cells in this form for years if put on filter paper into sealed glass tube.

II. Oxygen Requirements
A. classification by oxygen requirements
1. aerobes
    a. require oxygen in high concentration: 10-20%
2. microaerophiles
    a. require oxygen in low concentration: 2-10%
3. anaerobes
    a. obligate anaerobes
        1. can only grow w/o oxygen
    b. facultative anaerobes
        1. can grow in presence or absence of oxygen

B. why some species are obligate anaerobes
1. during electron transport, process of flavoprotein oxidation generates superoxide radical
a. superoxide radical is oxygen w/ an extra electron, so has net negative charge
b. it's highly reactive and damages DNA, proteins, and membranes- lethal to cell
2. superoxide dismutase is needed to degrade superoxide radical
a. carries out reaction: $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
3. catalase is needed to degrade hydrogen peroxide which is also lethal to the cell
a. carries out reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$
4. which types of bac have these enzymes?
a. aerobes have both enzymes, so can grow in a lot of oxygen
b. microaerophiles have dismutase, but not catalase
1. but they have peroxidase which breaks down peroxide, too, but more slowly
2. therefore, they can only survive in limited amounts of oxygen
c. obligate anaerobes do not have these enzymes, so die if grown in any oxygen

C. types of obligate anaerobes
1. spore-forming
a. Clostridium tetani
1. causes tetanus
2. produces neurotoxin which causes spastic paralysis
A. all muscles of body contract and can't relax
B. problem b/c diaphragm needs to move in order to breath
b. Clostridium botulinum
1. causes botulism
2. produces neurotoxin which causes flaccid paralysis
A. all muscles of body relax and can't contract
B. problem b/c diaphragm can't move
c. Clostridium perfringens
1. causes gas gangrene
A. org produce gas in muscles, causing muscle fibers to explode
2. comes from shrapnel, bullet wounds- used in military context
2. non-spore forming
a. bacteroides fragilis
1. most common org in gut/intestinal tract
b. fusiform bacteria
1. found in mouth- little pockets b/t teeth and under gums
2. cause infection after oral surgery when enter bloodstream

D. how to handle obligate anaerobes
1. how to transport sample from patient to lab
a. use anaerobic culturette
1. soft plastic tube w/ screw cap w/ swab attached to cap
2. after swabbing patient, cover tube tightly
3. break ampule filled with chemicals inside tube
4. this causes chemical reactions to remove oxygen from the tube
2. how to grow up org in the lab
a. use thioglycollate broth- thioglycollate removes oxygen from the broth
b. use gas pak if grow on solid media
1. put gas pak in w/ petri dishes in large chamber that seals tightly
2. add water to gas pak which starts reaction to use up oxygen in chamber

III. PH

A. w/ relation to growth rate

1. minimum
    a. if more acidic than that, it won't grow
2. maximum
    a. if more alkaline than that, it won't grow
3. range
    a. the pH at which bac can grow
4. optimum
    a. best pH for growth rate

B. classification by pH

1. acidophiles
    a. grow at pH 6.5-7
2. neutrophiles
    a. grow at pH 7-8
        1. most pathogens grow at pH 7.2-7.6 = pH of blood and body tissues
3. alkalophiles
    a. grow at pH 8.4-9
4. fungi
    a. grow at pH 4-6
5. extreme cases
    a. Acetobacter
        1. grows at very low pH: 2-3
    b. vibrio cholerae
        1. grows at pH 9 = very alkalinic
        2. used in diagnostic microbio- if org grows at high pH, it's probably vibrio

C. how pH affects growth rate

1. mechanism: $AH \leftrightarrow A^- + H^+$
    a. a high concentration of H+ will push the reaction backward to form AH
    b. cell is more permeable to the undissociated form of the molecule b/c it's neutral
    c. the undissociated form is more inhibitory to growth of bacterial cells
    d. H+ itself also inhibits enzyme activity b/c being far from optimum pH weakens enzyme structure, inhibiting bac growth

D. how to regulate pH- maintain homeostasis

1. transport of protons
    a. pump in protons if pH too high, pump out protons if pH too low
2. conversion of acidic molecules into neutral molecules to raise pH of the cell
    a. occurs in Enterobacter, Bacillus, Klebsiella, and Serratia
    b. ex: is conversion of 2 pyruvic acids (acidic) into acetolactic acid into acetoin into butanediol (neutral). See your own diagram.
    c. 2 pyruvic acids are converted into acetolactic acid by removing a carboxyl group and by breaking double bond of carbonyl group. Then it's converted into acetoin by removing 2nd carboxyl group. Then it's converted into butanediol by breaking double bond of 2nd carbonyl group.
3. induction of polyamine synthesis
    a. switch is turned on to make mRNA to make amino acid decarboxylases
    b. ornithine decarboxylase turns ornithine (a 4 carbon amino acid) into putrescine (a polyamine- more alkaline) by removaing a carboxyl group. See your own diagram.
    c. lysine decarboxylase turns lysine (a 5 carbon amino acid) into cadaverine (a polyamine- more alkaline) by removing a carboxyl group. See your own diagram.

d. 2 impt points:

1. diagnostic microbio- can see if cell has ODC or LDC to tell what org it is
2. pathogens that get past HCL of the stomach and stays in intestines must have amino acid decarboxylases to raise pH in the cell to survive

IV. Osmolarity

A. refers to salt concentration

1. it's a colligative property- a function of the number of particles, regardless of the nature of the particles

a. salt conc= conc of amino acids, metabolic intermediates, vitamins...not just salts

B. types of environments

1. isotonic solution
   a. salt conc in and out of cell is equal- ideal condition
2. hypotonic solution
   a. extreme of low salt outside = plain water
   b. water will move inside the cell, causing the cell to swell and burst- lysis
3. hypertonic solution
   a. water will move outside cell, causing cell membrane to shrink- crenation
   b. exception = halophiles- marine bac, live in salty oceans

C. how bac survive low salt environment

a. generally cell wall protects against lysis

b. there are special cases:

1. protoplasts and spheroplasts- cells lost cell walls
2. mycoplasma- species which doesn't have a cell wall
3. w/o cell wall, they're osmotically fragile
   A. antibiotics makes cell osmotically fragile by inhibiting cell wall synthesis
   B. if patient has pneumonia caused by mycoplasma, penicillin won't work b/c no cell wall, so can't inhibit cell wall synthesis

D. how to regulate osmolarity to maintain homeostasis

1. cytoplasmic osmoregulation
   a. increase or decrease potassium ions
   b. increase or decrease polyamine synthesis
      1. this makes more or less molecules in the cell to regulate osmotic pressure
   c. synthesis or uptake of osmoprotectants
      a. ex: proline (amino acid) and betaine (molecule that is a modified version of trimethylglycine, an amino acid)
      b. stabilize proteins at high salt
2. periplasmic osmoregulation
   a. uses MDO= membrane derived oligosaccharide
      1. 6-10 glucose molecules to which is attached succinate or phosphoglycerol- negatively charged groups
      2. they attach sodium ions to bring more into the cell
   b. MDO is made in the cytoplasm and put more or less in periplasmic space, depending on what is needed- more or less sodium ions
   c. if medium salt conc is low, MDO conc in periplasmic space is high b/c salt in cell is high and MDO fools cell mem into thinking environment is high salt also
   d. if medium salt conc is high, MDO conc in periplasmic space is low b/c salt in cell is low and MDO fools cell mem into thinking environment is low salt also

V. Moisture
- A. bac require 95% moisture
  - 1. bac on surface of skin do not grow, but are viable
  - 2. if there's a break in the skin and surface becomes moist, bac can grow and cause infections
- B. yeast need 75%
- C. fungi need 50%
  - 1. can grow on places on body that have some moisture, but not enough for bac
  - 2. fungi grow b/t toes, armpits...leading to fungal infections, such as Athlete's foot

VI. Pressure
- A. talking about bac growing on the bottom of the ocean: 1,000-11,000 m deep
  - 1. pressure at bottom of ocean is 1,000 atm
- B. classification by pressure
  - 1. barotolerant
    - a. survive pressures up to 400 atm, but grow best at 1 atm
  - 2. barophiles
    - a. prefer higher pressures
    - b. 2 types:
      - 1. moderate- grow best at high pressure
      - 2. extreme- grow only at high pressure
- C. study of bac in outer space
  - 1. want to know the effect of zero gravity on bac growth, morphology, pathogenesis, etc.
  - 2. company has device that enables us to study bac at zero gravity here on earth
- D. new field of study: extremophiles
  - 1. if bac can survive dry, saltless, anaerobic conditions on Earth, maybe there's life on Mars

VII. Sound
- A. ranges of sound waves
  - 1. auditory- up to 50,000 Hz
  - 2. supersonic- up to 200,000 Hz
  - 3. ultrasonic- more than 200,000 Hz
- B. can generate ultrasonic sound waves in solution, producing microscopic air bubbles, causing lysis
  - 1. process of producing air bubbles = cavitation
  - 2. used as an ultrasonic cleaner
    - a. removes lipids and proteins from tools- does not sterilize though
    - b. then can sterilize tools with disinfectant b/c disinfectant can now reach tools

VIII. Bacteria in the Body and Food Preservation
- A. temp
  - 1. body
    - a. fever raises temp, but can fry brain, so take Aspirin or Ibuprofen
  - 2. food
    - a. pasteurization of milk
      - 1. standard: raise temp to 62 degrees Celsius for 30 mins
      - 2. flash: raise temp to 75 degrees Celsius for 30 sec
    - b. use fridge to inhibit bac growth and preserve food
- B. pH
  - 1. body
    - a. few infections in stomach and intestines b/c too acidic for growth
  - 2. food
    - a. can put food in vinegar b/c acidity inhibits bac growth and preserves food

C. oxygen
    1. body
        a. anaerobes grow once penetrate the skin and grow in areas of the mouth and wounds
    2. food
        a. vacuum-pack food which preserves food and inhibits bac growth of aerobes
D. osmolarity
    1. food high in salt inhibits bac growth and preserves food
        a. ex: pickles, olives in brine (salt water)
    2. food high in sugar inhibits bac growth, too, b/c colligative property
        a. ex: jellies and jams
E. moisture
    1. dried fruits last for a long time
        a. raisins vs. grapes, prunes vs. plums
F. protective surface
    1. peels of fruits protect (ex: bananas and oranges)
    2. less surface area protects
        a. ex: chunk of meat more protected from bac than ground meat b/c less surface area