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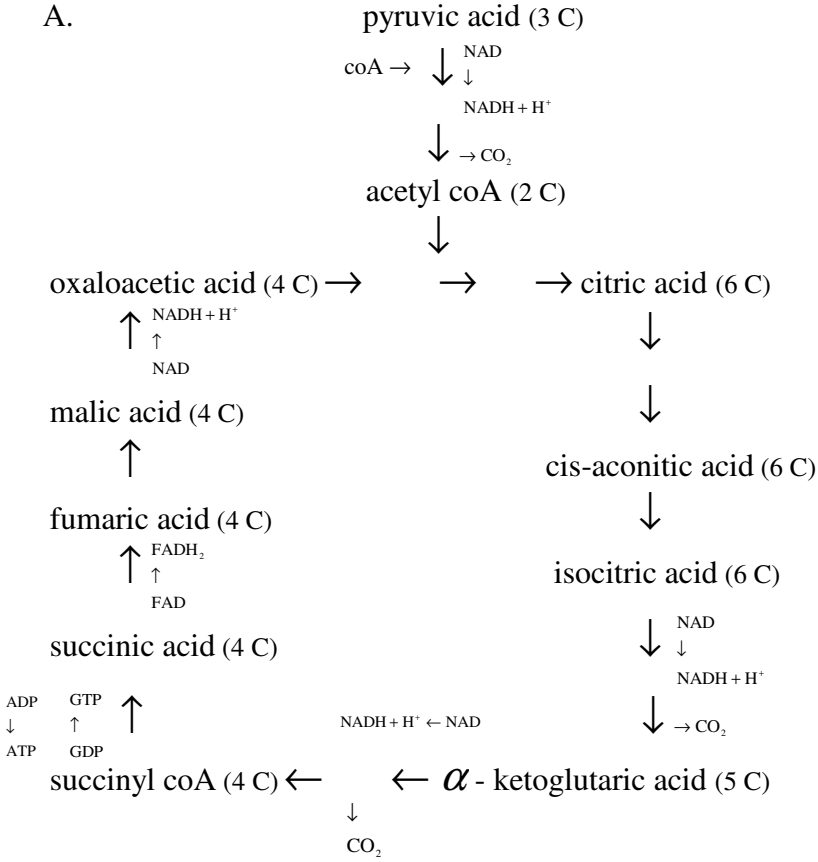
Energetics- Microbiology Test 4B

I. General Overview of Respiration

A.



B. FAD=flavin adenine dinucleotide

1. it picks up 2 protons and becomes $FADH_2$

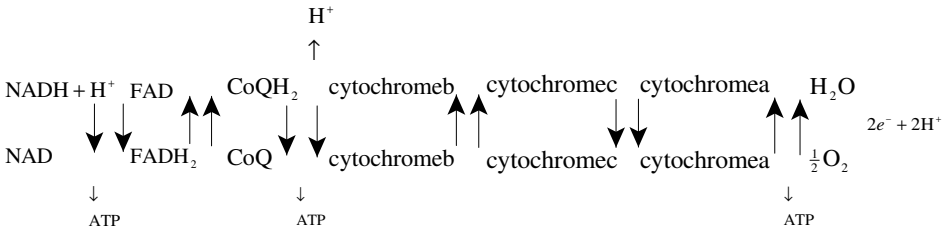
C. succinyl coA is a high energy molecule

1. it provides energy for phosphorylation of GDP to make GTP
2. that energy is then transferred to ADP to make ATP

II. Aerobic Respiration=Electron Transport System

A. we're taking protons and electrons from NADH and passing them on within the cell membrane

B.



C. from CoQ —> cytochrome b, protons are being pumped outside the membrane and only electrons are being passed on

D. oxidative phosphorylation = synthesis of ATP

1. occurs at 3 different locations during the electron transport system

a. $NADH + H^+ \rightarrow NAD$ - makes 1 ATP

b. $CoQH_2 \rightarrow CoQ$ - makes 1 ATP

c. cytochrome a (reduced) ⟶ cytochrome a (oxidized)- makes 1 ATP

E cytochrome oxidase is the enzyme that catalyzes the last step (cyt a —> water)

F. from $NADH + H^+$, you get 3 ATP and from $FADH_2$, you get 2 ATP

G. end goal of glycolysis: taking energy from chemical bonds of glucose and energy from electrons and putting them on various molecules- making ATP

H. at each step of ETS, you're losing energy and that provides the energy to pump out the protons.

I. when protons return back into the cell, that proton motive force provides the energy for the synthesis of ATP (energizes the enzyme that converts ADP to ATP) at the 3 places where ATP is produced in ETS

1. that energy can also be used to actively transport substrates into the cell, such as lactose

III. Anaerobic Respiration

A. it's the same thing as aerobic respiration, except oxygen is not the terminal electron acceptor

1. goes through electron transport system and has oxidative phosphorylation

B. instead of oxygen, receptors can be NO_3, NO_2, SO_3, CO_3, fumurate, or iron

C. the enzyme that helps pass the electrons to the terminal acceptor (last step in ETS) is terminal oxidase

IV. Fermentation

A. growth in the absence of oxygen, but no electron transport system or Krebs cycle

B. the step in b/t alpha-ketoglutaric acid and succinyl coA is inhibited b/c no electron acceptor

1. this stops Kreb's cycle

2. steps from succinyl coA to oxaloacetic acid are reversed to get 4C intermediates for biosynthesis

C. the step from pyruvic acid to acetyl coA is also inhibited b/c no electron acceptor

1. this causes buildup of pyruvic acid from glycolysis

2. there are a number of possible fermentation patterns possible that occur in different organsims

a. lactic fermentation

1. pyruvic acid $\longrightarrow$ lactic acid

2. ex: in streptococcus and lactobacillus

b. alcoholic fermentation

1. pyruvic acid $\longrightarrow$ acetaldehyde $\longrightarrow$ ethanol

2. ex: in yeast (eukaryotic org, fungus- not bac)

A. used by manufacturers of beer and wine

c. mixed acid fermentation

1. 2 processes:

A. pyruvic acid $\longrightarrow$ formic acid $\longrightarrow$ $H_2(g) + CO_2(g)$

B. pyruvic acid $\longrightarrow$ acetyl coA $\longrightarrow$ acetic acid $\longrightarrow$ ethanol

2. ex: in enteric bacteria- bac found in the intestines (E. coli, salmonella, shigella)

3. can use this for diagnostic microbiology- can set up test for acid production

A. not every org produces acid, but enteric bac produce a lot

d. butanediol fermentation

1. pyruvic acid $\longrightarrow$ acetoin $\longrightarrow$ butanediol

2. ex: in enterobacter

A. resembles E. coli, but normal for it to be found in water supply

B. when test water for enteric bac such as E. coli, need to be able to tell dif

C. test for butanediol fermentation- only enterobacter produces it

e. butyric fermentation

1. pyruvic acid $\longrightarrow$ acetyl coA $\longrightarrow$ acetoacetic acid $\longrightarrow$ butyric acid $\longrightarrow$ butanol, acetone, or isopropyl

2. ex: in clostridia

3. played critical role in the founding of the state of Israel b/c Chaim Weissman discovered butyric fermentation. Acetone is ingredient in gunpowder which helped manufacturers of gun shells which helped us win WWI which helped in formation of state of Israel.

f. propionic fermentation

1. pyruvic acid → oxaloacetic acid → succinic acid → propionic acid
2. ex: in propioni bacteria

D. can have 3-story fermentation tank and grow up many bac, each producing different products...

1. can grow lots of bac to make product we want

V. Substrate Level Phosphorylation

A. this is the way bac can make ATP even when growing w/o oxygen (fermentation)

B.

step in glycolysis	ATP used	ATP produced	net
glucose → glucose-6-phosphate	1		
fructose-6-phosphate → fructose 1,6-diphosphate	1		
1,3-diphosphoglyceric acid → 3 phosphoglyceric acid		2	
phosphoenol pyruvic acid → pyruvic acid		2	
	2	4	2 ATP produced

C. these 2 ATP provide the energy for fermentation

VI. Energy for Respiration

A.

ATP-producing step	ATP produced for that step	how many times that step occurs	total ATP produced due to that step
glyceraldehyde-3-phosphate → 1,3-diphosphoglyceric acid during this step, NAD comes in and NADH comes out	3	2	6
pyruvic acid → acetyl coA during this step, NAD comes in and NADH comes out	3	2	6
NADH in Kreb's	3	6 (3 per cycle, 2 cycles)	18
$FADH_2$ in Kreb's	2	2 (1 per cycle, 2 cycles)	4
GTP → ATP	1	2 (1 per cycle, 2 cycles)	2
substrate level phosphorylation (from before)	2		2
Total			38

B. get 38 ATP molecules per molecule of glucose from respiration (only 2 ATP mol from fermentation)

C. bac take the energy stored in the bonds of glucose and store it as ATP

D. bac that grow using respiration have increased growth rate and yield (compared to fermentation)

VII. Different Types of Media
- A. liquid media (broth)
  1. can get a large number of cells
  2. can use it for a study- get info about rate and yield from growth curve
- B. solid media- plates
  1. can use it to isolate pure cultures- streak for single colonies
  2. can enumerate colonies (serial dilution)
  3. can determine morphology of a colony
  4. can observe certain properties, such as swarming
- C. solid media- lawn procedure (spread bac with hockey stick)
  1. growth factor
     - a. plate is not sufficient for growth
     - b. put growth factor on disk on medium
       1. factor will diffuse from disk to medium
       2. if get growth around disk, the growth factor can support growth
  2. antimicrobial sensitivity
     - a. use medium that can support bac growth
     - b. put disk with antibiotics on the medium
       1. antibiotics will diffuse from disk to medium
       2. if it has an effect, will get a zone of inhibition around the disk
          - A. there will be lawn everywhere around the zone of inhibition
  3. phage sensitivity
     - a. bac phage is a bac virus
       1. phage is just DNA inside a protein coat
       2. it lands on surface of bac and injects its DNA into cell
          - A. there's specificity- specific proteins on phage must bind to specific receptors on bac cell
            1. phage typing- by knowing which bac a phage will infect, can id unknown org
          - B. this causes bac cell to...
            1. make copies of the phage DNA
            2. make coat proteins that wrap around DNA of phage (assembly)
               - a. one phage causes bac to make 100 phage particles
          - C. then there is lysis of bac cell and phage particles are released
            1. the 100 particles can affect 100 more cells
          - D. phage can now infect bac cell and start process all over again
     - b. add phage to medium- if plaque forms around phage, there was a successful infection
       1. plaque=clearing
       2. no bac b/c phage caused lysis of bac cells
- D. solid media- tubes/slants
  1. can see if bac will grow on this medium or not
  2. more convenient than plates
  3. can store them longer than plates (1-2 months vs. 1-2 weeks for plates)
  4. can stab tube to see if aerobic or anaerobic depending upon where growth occurs
     - a. facultative anaerobes- growth occurs along the entire stab line
     - b. aerobes growth only occurs at top of tube
     - c. obligate anaerobes- growth only occurs at bottom of tube
  5. can stab tube of semi-solid agar to test for motility
     - a. non-motile- growth only occurs along stabline
     - b. motile- growth occurs throughout the tube

VIII. Different Purposes of Media
- A. enrichment media- used for growth of fastidious organisms, for a good yield
  - 1. trypticase soy broth
    - a. use trypsin to digest meat and add digest to make rich medium
  - 2. nutrient broth
    - a. ex: beef extract- boil beef, extract nutrients, and use them to supplement medium
  - 3. blood agar
    - a. contains beef heart muscle and 5% blood- usually sheep's blood
  - 4. chocolate agar
    - a. comes from boiled blood
    - b. used for isolating dif org that can grow on this agar:
      1. hemophilus- causes the flu
      2. neisseria- causes gonorrhea or meningitis
      3. legionelle- causes legionairre's disease (respiratory disease)
    - c. impt ingredients:
      1. protease peptones
      2. yeast concentrate
      3. NAD
      4. hemin (blood derivative)
- B. selective media- when have mixed culture, this media is used to favor the org you want
  - 1. use minimal medium
    - a. excludes fastidious org
  - 2. add dye or chemical to medium
    - a. inhibits growth of some bac and allows growth of others
      1. EMB agar
         - A. contains eosin and methylene blue
         - B. only allows G- org to grow
      2. phenyl ethyl alcohol agar
         - A. only allows G+ org to grow
  - 3. add antibiotics to medium
    - a. CNA agar
      1. contains colistin and nalidixic acid
      2. only allows G+ org to grow
    - b. Thayer-Martin agar
      1. chocolate agar with vancomycin, colistin, and nystatin- 3 antibiotics
      2. only allows neisseria to grow
  - 4. carbon sources
    - a. to isolate org, use carbon source that only that org can grow on as sole C-source
  - 5. factors
    - a. pH
      1. ex: vibrio grows at high pH, so plate w/ high alkalinity to grow vibrio
    - b. osmolarity
      1. if org can grow at high salt conc, plate w/ high salt to inhibit growth of other org
    - c. oxygen concentration
      1. if org can only grow under anaerobic conditions, you can inhibit growth of org requiring oxygen for growth (or vice versa)

C. differential media- used to differentiate b/t org based on particular properties
1. carbon source utilization
a. Is an exoenzyme produced?
1. starch agar lab test
b. Is the carbon source utilized?
1. use it as sole carbon source- see if org can grow or not using that source
2. use pH indicator dye
A. Simmon's citrate tube
1. contains citrate and dye
2. if org uses citrate as C-source, acid is produced
3. acid causes dye to change from green to blue
B. EMB plate
1. differentiates b/t org that are lac+ and lac-
2. lac=notation for gene that codes for enzyme that allows org to use lactose as C-source b/c enzyme splits the disaccharide lactose into 2 sugars
3. use this to test stool samples for enteric pathogens
a. E. coli- normal flora/non-pathogen- lac+
b. salmonella and shigella- pathogens- lac-
4. take EMB plate w/ high concentration of lactose and dye
a. lac+ org use lactose as C-source
1. since enteric org use mixed acid fermentation, this produces acid
2. this causes dye to change color, giving colony a dark spot
b. lac- org can't use lactose
1. it will grow, but won't produce enough acid b/c not using lactose
2. no color change will take place

2. pathway utilization
a. if org grows when only carbon sources are 5 carbon sugars, org must have functioning pentose cycle
b. if org grows on citrate or succinate as sole carbon source, org must have functioning Kreb's cycle
c. electron transport analysis
1. to determine if oxygen is the terminal acceptor...
A. use tetramethyl phenylene diamine=cytochrome oxidase reagent
B. add a few drops of this reagent to plate
C. if there's a color change, know cytochrome oxidase is present
D. therefore, know oxygen is the electron acceptor
2. to determine if tellurium is the terminal acceptor...
A. add tellurite to the plate
B. if get a black colony, know tellurium is the electron acceptor
3. to determine if iron is the terminal acceptor...
A. add Fe^{3+} (ferric ion) and a sulfur-containing compound to a plate
B. if black precipitate forms, know its FeS (ferrous sulfide)
C. this means iron ion gained an electron, so it's the electron acceptor for that org

3. product formation
    a. hemolysin production
        1. hemolysin - toxin which destroys red blood cells
        2. use blood agar plate to see if hemolysis occurs
        3. this shows up as a clearing around the bac colony- no blood in agar there
            A. α -hemolysis- partial clearing, has greenish coloration
                1. find this with non-pathogenic streptococcus
            B. β -hemolysis- complete clearing
                1. find this with pathogenic streptococcus- can cause heart or kidney disease
    b. decarboxylase production
        1. put a high conc of substrate (lysine, ornithine...) and pH indicator dye in medium
        2. if a decarboxylase enzyme is present, the dye will change color from yellow to purple
            A. medium will become more alkaline b/c polyamines are formed
                1. lysine decarboxylase converts lysine into cadaverine
                2. ornithine decarboxylase converts ornithine into putrescine
        3. if decarboxylase enzyme is not present, there will be no color change
    c. triple sugar iron (TSI) tubes
        1. contain glucose (1X), lactose (10X), sucrose (10X), iron, and dye
        2. used to differentiate b/t lac+ and lac- organisms
            A. streak surface of slant and stab into butt
            B. if tube red (alkaline) by slant and yellow (acidic) by butt, org is lac-
                1. since can't use lactose, org growing by respiration on surface, producing some acid, but not enough to change basic red to acidic yellow
                2. but in the butt, org is growing by mixed acid fermentation, producing a lot of acid, causing the color change to yellow
                3. lac- can be salmonella or shigella
            C. if tube is yellow (acidic) by both slant and butt, org is lac+
                1. org can use lactose, so produces a lot of acid even at the top, causing a color change
                2. E. coli is lac+
        3. once it is clear org is lac-, can differentiate b/t salmonella and shigella
            A. if tube is red by slant, yellow by butt, and has black precipitate, it's salmonella
                1. this is b/c salmonella produces H_2S
                2. it reacts w/ Fe to produce FeS- a black precipitate
            B. if tube shows lac- bac, but no black precipitate, it's shigella
        4. if tube is red by both the slant and butt, it means no acid is produced
            A. this is b/c the org uses Entner-Deudoroff pathway, such as pseudomonas