

Review sheet for MCB235/336

15 questions from Part I will constitute the Third Exam

15 questions from Part II will constitute the Final Exam

PART I – Third Exam Prep. - May be more than one answer

1. Which of the following are ways that ascorbic acid is produced industrially?

- The Holstein-Bungy process.
- By the Sonoyama process using two organisms to carry out partial oxidations.
- By the Reichstein-Grussner process.
- By recombinant organisms that convert glucose to 2-keto-L-gulonic acid.
- By biotransformation of sorbitol by *Gluconobacter oxydans*.

2. Match the following organisms with the product:

<i>Ashby gossypii</i>	Vitamin C
<i>Blakeslea trispora</i>	Riboflavin
<i>Propionibacterium shermanii</i>	Vitamin B12
<i>Gluconobacter oxydans</i>	Vitamin A
<i>Erwinia herbicola</i>	β -carotene

3. The best raw material for making progesterone was found to be:

- Ox bile
- Carrots
- The fungus *Ashby gossypii*
- Mexican Barbasco yams
- Agave plants

4. How did the introduction of microorganisms lower the price for making cortisone and related steroids?

- Steroids extracted from *Mucor* proved more abundant than those from ox adrenals
- Fungi released steroids from plants, thus making synthesis easier and cheaper.
- Mucor* stereospecifically added an OH- group to the steroid nucleus at position 11.
- The fungus made cortisone without further chemical reactions.
- Streptomyces* make the precursor to cortisone from corn steep liquor.

5. William Castle demonstrated that:

- Intrinsic factor was needed to absorb vitamin B12
- Ascorbic acid could be made in a single step process
- Pepsin could be used as a soft drink
- Takadiastase was good for the digestion
- Regurgitated meat was a cure for vegetarianism.

6. Leader sequences in protein export:

- Form pores in the cytoplasmic membrane through which the proteins pass.
- Bind to ribosomes and stall translation.
- Tag proteins for export by the Sec system.
- Bind to the Signal Recognition Particle (SRP).
- Delay the folding of the whole polypeptide.

7. Most enzymes made on an industrial scale are:
- Hydrolases
 - Oxidoreductases requiring FAD or FMN
 - Involved in the TCA cycle.
 - Are exoenzymes
 - Are subject to nitrogen or carbon catabolite repression.

7a. What are the three major classes of industrial proteases? Give an example of the industries in which each class is used.

7b. Fill in the blanks with the appropriate enzyme, substrate or endproduct.

Enzyme	Substrate	End-Products
amyloglucosidase		
	starch	oligosaccharides
	oligosaccharides	maltose
		amino acids & oligopeptides
		coagulated milk

8. Where do fusel alcohols come from during the fermentation of beer?

- From ethyl alcohol.
- From hops used as a flavoring agent.
- From amino acids in barley.
- From the adjuncts.
- From transamination reactions.
- From an evil spirit that inhabits the brew kettle.

9. Beer fermentation is a large-scale industrial fermentation developed empirically through the centuries. What is the most likely reason that virtually all cultures managed to develop their own version of an alcoholic beverage.

- The need to develop a safe source of drink
- The lack of refrigeration
- The ubiquitous presence of wild yeast on grain and fruit
- The use of yeast for making leavened bread
- Ancient extraterrestrials giving each culture the recipe.

10. Which of the following statements about antibiotics is generally true?

- All antibiotics are made from common amino acids.
- The production of most antibiotics is subject to some kind of catabolite repression.
- Individual antibiotic-producing microorganisms often make several related antibiotics.
- Antibiotic biosynthetic pathways are generally very short involving one or two novel enzymes.
- All antibiotics are made on multi-component enzymes called polyketide synthases.

11. Which of the following is true about β -lactam antibiotics?

- Penicillin-G is a β -lactam antibiotic.
- β -lactam antibiotics are rarely used today and are mainly of historical interest.
- Only fungi make β -lactam antibiotics.
- β -lactam antibiotics are made from polyketide intermediates.
- Most β -lactam antibiotics used today are 'semi-synthetic'.

12. Peptide antibiotics are made by:
- Non-ribosomal peptide synthetases - NRPSs
 - Methanogens
 - Translation of short mRNAs
 - Sporulating bacteria
 - Methylotrophs
13. The class of secondary metabolites that currently have the largest market share are the:
- Ansamycins
 - Polyene macrolides
 - Aminoglycosides
 - β -lactams
 - Tetracyclines
14. Identify the organism that produces each of the following antibiotics:
- Cephalosporin C _____
 - Penicillin N _____
 - Tetracycline _____
 - Streptomycin _____
 - Erythromycin _____
15. What is the role of clavulanic acid in the drug Augmentin[®]?
- It is a growth promotant used to make bigger cows.
 - It is a 'semisynthetic' antibiotic.
 - It inhibits penicillinases.
 - It blocks cell wall synthesis and thus kills pathogens.
 - It prevents tissue rejection in organ transplant patients.
16. Although structurally very different, tetracycline and erythromycin both...
- ...are made from amino acids.
 - ...are made via polyketide intermediates.
 - ...are built from short chain fatty acids esterified to CoASH.
 - ...block protein synthesis on the bacterial ribosome.
 - ...are made by actinobacteria.
 - ...are made by non-ribosomal peptide synthetases.
17. What do ampicillin, carbenicillin, and methicillin have in common?
- All are products of "directed biosynthesis"
 - 6-APA
 - 7-ACA
 - All are 'semisynthetic' antibiotics
 - All are β -lactam antibiotics

18. Provide the two reasons most often given for why antibiotic-resistant organisms in the environment and in hospitals have become a major problem.

a.

b.

19. R-factor or plasmid-mediated resistance to antibiotics...

- a. ...is usually characterized by modification or inactivation of an antibiotic.
- b. ...can spread rapidly in bacterial populations.
- c. ...generally leads to a mutation in a gene encoding the target of an antibiotic (e.g. ribosomal protein).
- d. ...occurs naturally at low levels in bacterial populations that have no previous exposure to an antibiotic.
- e. ...is the leading cause of antibiotic resistance in hospitals.

20. Cyclosporin A:

- a. Is produced by _____.
- b. Was originally studied by a scientist named _____.
- c. Acts as a _____.
- d. Has led to an increase in the number of _____.

21. Ivermectin:

- a. Is produced by _____.
- b. Was originally made by a company named _____.
- c. Has its major effect on _____.
- d. Has led to a cure for _____.

22. Complete the following chart:

Antibiotic	Target in cell	Producing organism	Class of Antibiotic
Streptomycin			
Chlorotetracycline			
Clavulanic acid			
Erythromycin			
Amphotericin B			

23. Define:

a. Mutational Biosynthesis

b. Protoplast fusion

24. Chromosomal antibiotic resistance...

- a. ...is not inherited by progeny cells.
- b. ...results in target modification.
- c. ...can occur at a frequency corresponding to the spontaneous mutation rate of the cell.
- d. ...is an active process carried out by a few pathogenic bacteria.
- e. ...is 'passive' due to mutation of a normal cellular gene.

25. Polyketide synthetases are large enzymes that put together a wide variety of secondary metabolites including:

- a. tetracyclines
- b. penicillins
- c. aminoglycosides
- d. polyene macrolides
- e. macrocyclic lactones

26. Polyketide synthetases seem to have been derived, evolutionarily, from:

- a. ribosomes
- d. peptidoglycan biosynthetic enzymes
- c. non-ribosomal peptide synthases
- d. fatty acid biosynthetic enzymes
- e. starch-degrading enzymes

27. Define:

Antibiotic

Antimetabolite

Antimicrobial agent

28. In what way do Selman Waksman and Gerhard Domagk represent two different approaches to the discovery of antimicrobial agents?

29. What are four approaches used for identifying novel antimicrobial agents? Provide an example of how have they been used with success in recent years.

30. Draw the structure of Penicillin G, 6-APA and 7-ACA.

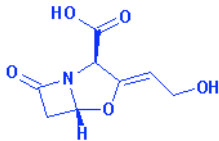
31. Cephalosporins emerged in the 1960s as effective antibiotics and alternatives to penicillins. What do the two classes of antibiotics have in common?

- a. Both are produced by the same microorganism.
- b. Both interfere with the biosynthesis of peptidoglycan.
- c. Both have a β -lactam ring that is responsible for their activity.
- d. Both are made by the essentially the same biochemical pathway.
- e. Both are polyketide antibiotics.

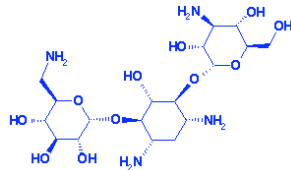
32. Cephalosporin C...

- a. ...was discovered by Alexander Fleming.
- b. ...is the first "cephalosporin" discovered.
- c. ...contains 7-ACA as part of its structure.
- d. ...was first brought to market by Eli Lilly Co. even though it was discovered in Italy.
- e. ...was initially produced by *Penicillium chrysogenum*.

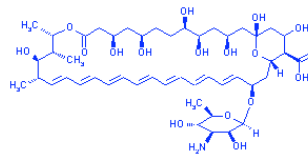
33. Identify the major class of antibiotics to which the following belong:



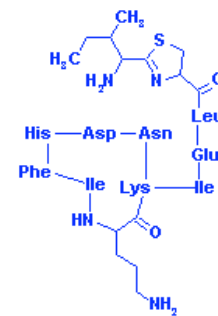
a.



b.



c.



d.

34. Vancomycin resistant enterococci emerged relatively recently in Europe. What event is thought to have promoted their emergence into the population?

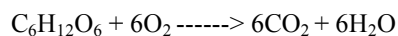
- a. Overuse of vancomycin by hospitals for trivial illnesses.
- b. Evolution of a new type of bacterial cell wall that no longer binds vancomycin.
- c. Evolution of a new type of bacterial ribosome that no longer binds vancomycin.
- d. Overuse of a related antibiotic as a food additive by European farmers.
- e. Increase in the availability of vancomycin "over-the-counter" in European apothecaries.

35. Which of the following are characteristics of vancomycin:

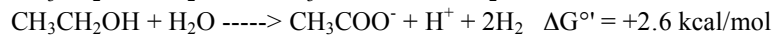
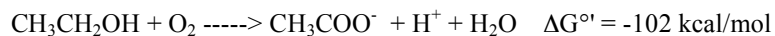
- a. It is an older antibiotic that was initially ignored because some preparations were considered too toxic.
- b. It is made by the actinobacterium *Amycolatopsis orientalis*.
- c. It cannot be used for severe infections by methicillin resistant *Staphylococcus aureus* (MRSA).
- d. It is one of the few polyketide antibiotics made by a single enzyme.
- e. It is used as one of the few effective antifungal antibiotics.

36. Nonribosomal peptide synthases generally are required to make which of the following
- peptide antibiotics.
 - β -lactam antibiotics
 - polyketide intermediates
 - aminoglycoside secondary metabolites
 - fluoroquinolones
37. The global C-cycle generally derives its energy for operation ultimately from:
- Methanogenesis
 - Respiration
 - Light
 - Fermentation
 - Methane oxidation
38. The free energy change of a biological reaction (ΔG) and the change that occurs in redox potential are...
- ...directly related by a constant.
 - ...not related since one is logarithmic and the other is linear.
 - ...inversely related to the number of electrons transferred.
 - ...what determines if a reaction will be "spontaneous".
 - ...different ways of expressing the extent and direction of a reaction.
39. The "Midpoint potential" of a compound involved in oxidation-reduction is:
- The point at which the pair is halfway up a redox tower.
 - The actual redox potential of a compound in a natural environment.
 - Constant for each redox pair as compared to the hydrogen electrode.
 - The point at which a compound has an equal amount of oxidized and reduced species present.
 - The pH of a 1 M solution of a compound.
40. Which of the following determines if, and how fast, a compound will be biodegraded by microorganisms?
- The amount of energy released during the breakdown of the compound
 - The solubility of the chemical in water
 - Presence of branched chains on the molecule
 - Presence of tertiary amines
 - The presence of O_2 in the environment
41. The difference between anaerobic degradation in the rumen versus anaerobic sediments is:
- The absence of methanogens in the rumen.
 - The presence of cellulolytic organisms in the rumen but not in sediments.
 - The fate of volatile fatty acids like acetate, propionate and butyrate in the rumen versus sediments.
 - The population of microorganisms present in the rumen is unique.
 - Temperature
42. Identify the three general groups of organisms responsible for anaerobic degradation in sediments:
- -
 -

43. What do all petrochemical degrading biochemical pathways we described in class have in common?
- All are performed by one or more species of *Pseudomonas*.
 - All require O₂.
 - All lead to the production of acetate for entry into biochemical pathways.
 - None of the pathways would operate effectively in *Clostridium*.
 - All lead to βoxidation.
44. Reasons that biomagnification may occur include:
- the ability of microorganisms to convert a toxic compound to CO₂ and H₂O
 - the volatility of chlorinated hydrocarbons.
 - the solubility of some toxic compounds in lipids.
 - the recalcitrance of the toxic compound.
 - the increase in the bacterial population upon exposure to a new compound.
45. In Victorian London, sewage treatment consisted of a cesspool located in the basement of each house with an overflow emptying onto the street. This practice, combined with the custom of sealing the house at night to keep out the smoke from coal fires, led to unexplained deaths from asphyxiation. How would you explain such deaths to a baffled Victorian physician?
- Methanogenesis
 - Lactate fermentation
 - Stickland reaction
 - Methane oxidation
 - Anerobic respiration
46. What is the difference between ΔG° and ΔG°' in the following reaction:



- No difference
 - About 9.6 kcal/Mol
 - There is no way of knowing without the K_{eq} included
 - About 2.3 kcal
 - There is no way of knowing since this is not a redox reaction.
47. In class, we calculated the ΔG°' values for the catabolism of ethanol under anaerobic and aerobic conditions as follows:



Provide a thermodynamic reason for why more energy is available when ethanol is catabolized aerobically than anaerobically.

48. A good way to encourage microorganisms to degrade the components of crude oil in the soil environment is to:
- Flood the area in which the spill has occurred with water and cover it with plastic.
 - Steam clean the soil to dissolve the oil.
 - Till the soil to aerate.
 - Add nutrients such as iron, phosphorous or nitrogen.
 - Remove the soil to a landfill and bury.
49. If you were making a decision about whether to approve a new pesticide to sell the public, what are three of its chemical or biochemical characteristics that you would wish to know?
- -
 -
50. Biomagnification is a consequence of which of the following:
- Anaerobic degradation.
 - Aerobic degradation
 - Recalcitrance
 - Lipid solubility
 - UV polymerization

Part II – Final Exam Prep.

1. The history of applied microbiology is intimately intertwined with major political and social upheavals. Match the following developments with the person or historical event most often associated with the development (some matches may be multiple).
 - a. Large scale fermentation _____
 - b. Development of brewing and baking techniques _____
 - c. Discovery of penicillin _____
 - d. Discovery of microorganisms _____
 - e. Scientific explanation for fermentation _____
 1. Antonie von Leeuwenhoek
 2. Alexander Fleming
 3. Development of agriculture
 4. World War II
 5. World War I
 6. Louis Pasteur
2. **Taxonomy** comes from the Greek word *taxis* for "arrangement". In our context, it means the arranging of organisms. It has three parts, including:
 - a. Identification of an unknown organism using biochemical or molecular tests
 - b. Classification of an unknown organism to a group of similar organisms
 - c. Inventing a new name for an unknown organism
 - d. Applying a set of rules of nomenclature to name an organism
 - e. Isolating a new organism
 - f. Depositing all organisms into an internationally accessible culture collection
3. Suppose you isolated two organisms that were very similar in their biochemical and physiological profiles. Which one of the following would be necessary and sufficient for determining the extent to which they were related to each other.
 - a. Polyphasic classification schemes
 - b. DNA-DNA homology studies
 - c. 16S rRNA gene sequencing and comparison
 - d. Further staining and biochemical characterization
 - e. Listing the environments in which the organism is normally found
4. Carl Woese developed a phylogenetic rather than taxonomic approach to classifying microorganisms. Which of the following statements concerning his approach is correct?
 - a. His approach relies on comparing the sequences of ribosomal RNA molecules only.
 - b. His approach assumes that DNA or RNA can be used as a "molecular clock" where more changes in sequence imply greater evolutionary distance.
 - c. His approach requires that the whole genome of an unknown organism be sequenced before meaningful comparisons can be made.
 - d. His initial S_{AB} values were based on PCR amplification of 16S rDNA from organisms.
 - e. His work showed that the major groups of bacteria (Gram negative, Gram positive, cyanobacteria, and spirochetes) were "natural" groups whose members were phylogenetically related to each other.

5. Many research groups are addressing the issue of diversity of bacteria in soils or water by cloning and sequencing 16S rRNA genes directly from environmental samples. Why is it possible to draw conclusions about the metabolic diversity of bacteria using only fragments of DNA?
- Organisms with closely related 16S rRNA sequences usually have similar metabolisms.
 - All sequences isolated from the soil sample come from organisms that are already in culture.
 - Because all members of a bacterial genus have the same 16S rRNA sequence.
 - The sequences for 16S rRNAs of all bacteria are now available in databases.
 - It is not possible to draw any strong conclusions about metabolism from DNA sequences isolated from soil.
6. Consider a situation where three organisms are isolated and 16S rRNA oligonucleotide analyses are performed on them. In Woese's scheme, the pairs of organisms yield S_{AB} values of:

$$A \times B = 0.54$$

$$A \times C = 0.55$$

$$B \times C = 0.98$$

What can you conclude about this analysis?

- A, B & C are closely related and are probably in the same genus.
 - A & C and A & B are not closely related.
 - B & C should probably be in the same genus.
 - A & B are closely related.
 - The analysis is faulty.
7. All Proteobacteria:
- ...are able to grow using respiration and fermentation
 - ...use the Embden-Meyerhof-Parnas pathway for glucose catabolism
 - ...have a negative Gram staining reaction
 - ...have peptidoglycan in their cell walls
 - ...are pathogens
 - ...grow on sugars
8. "Pseudomonads" and related organisms were initially all included in the genus *Pseudomonas* but some recently have been split into other genera (*Burkholderia*, *Ralstonia*, *Delftia* etc.). What happened that led to their split into multiple genera?
- Large scale DNA-DNA reassociation experiments conducted in the 1960s separated them into several groups.
 - Pathogens were separated out into their own genus to avoid confusion in clinical situations.
 - 16S ribosomal RNA gene sequences clearly demonstrated that pseudomonads, as historically defined, fell into at least five clearly defined phylogenetic clusters.
 - Several "pseudomonads" were found to be Archaea and were renamed accordingly.
 - "Pseudomonads" were split into physiological groups based on their possession, or not, of the Embden-Meyerhof-Parnas pathway, and their ability to use methanol as a sole source of carbon and energy.
9. "Obligate aerobes" may be obligate because:
- Some lack fermentative enzymes like aldehyde and alcohol dehydrogenases.
 - Their glycolytic pathways yield insufficient ATP to support aerobic growth.
 - They only catabolize glucose via a mixed acid fermentation.
 - Some require molecular oxygen (O_2) as an essential part of their metabolism.
 - They lack the Tricarboxylic Acid Cycle as well as the Embden-Meyerhof-Parnas pathway.

10. Which of the following completes the definition of an 'obligate aerobe'.

"All obligate aerobes ...

- a. ...respire."
- b. ...use the Oxidative Pentose Phosphate pathway to catabolize glucose."
- c. ...require O₂ as terminal electron acceptor in electron transport phosphorylation."
- d. ...are Gram negative."
- e. ...can also ferment."

11. Which one of the following best completes the definition of an obligate anaerobe?

"All obligate anaerobes...

- a. ...also grow in the presence of O₂."
- b. ...respire nitrate."
- c. ...consume H₂ while making methane."
- d. ...ferment to gain energy by substrate level phosphorylation."
- e. ...cannot tolerate O₂ in their environment."

12. Which of the following completes the definition of a fermentative organism?

"All fermentative organisms...

- a. ...are obligate anaerobes."
- b. ...belong to a small group of closely related organisms."
- c. ...use an organic compound as both an electron acceptor and an electron donor."
- d. ...make ATP by substrate level phosphorylation only."
- e. ...are Gram negative."

13. Some activities are characteristic of microbial groups. For each activity listed below, provide a genus name for the organisms involved:

Genus

- a. Food poisoning _____
- b. Plant genetic engineering _____
- c. Petroleum degradation _____
- d. Exoenzyme production _____
- e. Root nodule formation _____
- f. Dairy fermentations _____

14. Sequencing of the *Pseudomonas aeruginosa* genome confirmed which of the following about the organism:

- a. It is very good at transporting and using many low MW compounds for growth.
- b. It is very good at producing methane.
- c. It has several fermentation pathways.
- d. It produces endospores.
- e. Its ability to resist antibiotics is due to numerous efflux systems.

15. A Gram negative, yellow-pigmented organism isolated from the vascular system of wilted plants, that is an obligate aerobic and that is oxidase and catalase positive is most likely a member of which of the following genera:
- Brassica* sp.
 - Streptomyces* sp..
 - Pseudomonas* sp
 - Escherichia* sp.
 - Xanthomonas* sp.

What does this organism produce that we eat? _____

16. *Pseudomonas*, *Zoogloea*, *Rhizobium* and *Xanthomonas* are all:

- Gram positive aerobes
- Capable of producing brominated aryl hydrocarbon pigments
- Plant pathogens
- Obligate aerobes
- Soil organisms
- Capable of catabolizing glucose through the Emden Meyerhof Parnas pathway.

17. Physiologically, species of *Gluconobacter* differ from *Acetobacter* as follows:

- Gluconobacter* prefers to grow on glucose whereas *Acetobacter* prefers ethanol.
- Gluconobacter* sp. are “underoxidizers”, *Acetobacter* sp. are “overoxidizers”.
- Gluconobacter* sp. lacks the pentose phosphate pathway, whereas *Acetobacter* has it.
- Gluconobacter* is spelled differently than *Acetobacter*
- Acetobacter* strains are used to make vinegar because they prefer to grow on ethanol.

18. The only organisms known to ferment sugars using the Entner-Doudoroff pathway are the:

- Lactic acid bacteria
- Pseudomonads
- Zymomonads
- Streptomyces
- Methane-oxidizing bacteria

19. The conversion of secondary alcohols to ketones, and primary alcohols to carboxylic acids can be mediated by:

- Mixed function oxygenases
- Polyketide synthases
- Acetic acid bacteria
- Dioxygenases
- Zymomonads
- Methanotrophs

20. Suppose you wished to isolate the following organisms. What would you include in the medium to select for their enrichment?

- | | |
|-----------------------------|-------|
| a. <i>Saccharomyces</i> | _____ |
| b. <i>Pseudomonas</i> | _____ |
| c. <i>Propionibacterium</i> | _____ |
| d. <i>Acetobacter</i> | _____ |
| e. <i>Methylomonas</i> | _____ |
| f. <i>Methanococcus</i> | _____ |

21. When growing on methane, obligate methanotrophs assimilate carbon when it reaches the oxidation state of:
- CH₄
 - CO₂
 - HCOOH
 - HCHO
 - HCHO and CO₂
 - CH₃OH and HCOOH
22. Why do facultative organisms like *E. coli* make fermentation products?
- To reoxidize reduced cofactors.
 - To use acids as “antibiotics” to limit the growth of other bacteria in the vicinity.
 - To rid cells of excess carbon that builds up during fermentation.
 - Their metabolism is inefficient during anaerobic growth, ie. the “glucose effect”
 - Because their usual metabolic pathways cannot function without oxygen.
23. In general, which of the following leads directly or indirectly to generating ATP during fermentation by the lactic acid bacteria:
- Nitrate reduction.
 - NADH reoxidation by flavoprotein oxidoreductases.
 - Citrate fermentation.
 - Arginine dihydrolase reaction.
 - Stickland reaction.
 - Ethanol rather than acetate production.
24. The pathway used by methylotrophs for carbon assimilation is called the _____. It is more/less (choose one) efficient than that used by the methanotrophs which use the _____ pathway.
25. Methanotrophs have found use in:
- Hydroxylation reactions often involving pollutant degradation.
 - Antibiotic biosynthesis
 - Marker organisms for petroleum and gas deposits
 - Methane production
 - Gasahol production from wood
26. “Rhizobia” were once thought to belong to a tight knit group of bacteria. Recent work has challenged that assumption by the discovery that certain methylotrophs and even a *Burkholderia* species can nodulate some legumes. What accounts for the apparent widespread ability of bacteria to nodulate legumes.
- The legumes apparently allow any Gram-negative organism to occupy their root nodules in the absence of a true “Rhizobium”.
 - Most root nodule bacteria are actually very closely related they just have different physiologies that do not relate to their phylogeny.
 - Occasionally other bacteria “sneak into” root nodules while the real rhizobia do the signaling with nodulins.
 - The *nod* genes, and not the organisms in which the genes reside, determines whether a symbiosis will be initiated.
 - The closely related *Agrobacterium* coinfects roots to allow gene transfer to take place.

27. The signal exchange between a “rhizobium” and a legume involves:
- Acetosyringone and bacitracin
 - Xanthomonadin and leghemoglobin
 - T-DNA and luteolin
 - Nod factors and flavonoids
 - pSyms and Ti plasmids
28. Why does *Agrobacterium* provide an ideal tool for the genetic engineering of plants?
- It is a virus that can infect the plant cell nucleus.
 - It forms root hair "infection threads" that direct the bacterium to the plant cell nucleus.
 - Once DNA is transferred, the plant shows no symptoms that would make it unmarketable.
 - Tumor genes in the T-DNA can be replaced by genes of economic value.
 - It confers on plants the ability to use luteolin to stimulate growth.
29. What is a Ti plasmid?
- The tumor-inducing plasmid of *Rhizobium*
 - The crown gall-producing plasmid from *Agrobacterium*.
 - A megaplasmid used for plant transformation.
 - A plasmid carrying the genes for symbiotic nitrogen fixation.
 - A large plasmid that replicates by the Windsor knot mechanism.
30. Which of the following characterizes “enteric” organisms?
- All enterics are closely related to the extent that some genera could be combined.
 - All enterics are obligate aerobes that dwell in the intestines of warm-blooded animals.
 - All enterics can ferment and respire.
 - Enterics are distinguished partly by the mix of fermentation products they make.
 - There is only one strain known for each species of enteric, for example, *E. coli* K12.

What does the word "enteric" mean? _____

31. How do facultative anaerobes such as *E. coli* continue to make TCA cycle intermediates for biosynthesis during anaerobic growth even though the TCA cycle generates lots of NADH?
- All TCA enzymes are repressed. Intermediates are assimilated from the growth medium.
 - All TCA enzymes are repressed. New pathways are induced to make α -ketoglutarate and succinyl-CoA.
 - Some enzymes are repressed, including succinate and α -ketoglutarate dehydrogenases.
 - Pyruvate dehydrogenase is replaced by Pyruvate-formate lyase to avoid making NADH.
 - The "cycle" is branched. The oxidative branch makes α ketoglutarate and a reductive branch makes succinyl-CoA.
32. In general, the butanediol fermentation differs from the mixed acid fermentation in which of the following ways:
- It is a result of the Entner-Doudoroff pathway.
 - It yields much more acid than the mixed acid fermentation.
 - It yields much more gas than the mixed acid fermentation.
 - It yields more neutral end products.
 - It has the potential to yield more ATP than the mixed acid fermentation.

33. Which of the following enterics are important food-poisoning organisms:
- Burkholderia*
 - Salmonella*
 - Shigella*
 - Erwinia*
 - Proteus*
 - Escherichia*
34. The “Low G+C Gram Positive” bacterial group is also known as the:
- Firmicutes
 - Actinobacteria
 - Clostridia
 - Proteobacteria
 - Mollicutes
35. Why are lactic acid bacteria selected for growth in milk during dairy fermentations?
- Most lactic acid bacteria do not require iron for growth and milk is poor in available iron.
 - Lactic acid bacteria prefer to ferment sugars; milk contains lots of lactose.
 - Lactic acid bacteria require many growth factors such as vitamins, amino acids, etc., which are abundant in milk.
 - Lactic acid bacteria require citric acid in order to operate the citric acid cycle; milk contains abundant citrate for this purpose.
 - Lactic acid bacteria require an alkaline pH in which to grow and milk is very basic.
36. Mannitol dehydrogenase, flavoprotein oxidoreductase and citrate fermentation, are all means used by some lactic acid bacteria to:
- Generate NADH for fermentation.
 - Reoxidize NADH to NAD⁺
 - Synthesize antibiotics
 - Develop antibiotic resistance
 - Increase their Y_{ATP}
37. The buttery taste of many dairy products is a result of:
- The formation of acetoin by fermentation of citrate.
 - The fermentation of lactose to lactic acid by *Streptococcus* sp.
 - Traces of butyric acid formed by the butyric acid bacteria.
 - Formation of diacetyl by citrate-fermenting lactic acid bacteria.
 - Traces of propionic acid produced by *Propionibacterium*..
38. Homofermentative lactic acid bacteria differ from the heterofermentative types by....
- Producing almost twice as much ATP per glucose fermented
 - Producing almost twice as much lactic acid as the heterofermentative organisms
 - being coccus shaped in contrast to the heterofermentative rod shape.
 - belonging to the genus *Streptococcus* and *Escherichia*.
 - Possessing the enzyme phosphoketolase.

39. If a bacterium produces heat-resistant endospores, that organism also:
- Belongs to the low G+C Gram positive lineage of Bacteria
 - Makes spores that contain dipicolinic acid.
 - Has a Gram negative cell wall.
 - Is an obligate aerobe
 - Exhibits some degree of filamentous growth.
40. Clostridia are often grouped into a few physiological groups. They include the:
- Methanogens
 - Methylomonads
 - Butyric acid bacteria
 - Amino acid fermenters
 - Acetogens
 - Lactic acid bacteria
41. Why do the amino acid fermenting clostridia carry out the Stickland reaction?
- To initiate sporulation.
 - To ferment glucose to acetic acid.
 - To reoxidize NADH.
 - To generate ATP.
 - To remove acid that inhibits their growth.
42. *Clostridium* spp. have been associated with warfare of various types and for various nefarious purposes. Which of the following have been used for such purposes:
- C. perfringens*
 - C. sporogenes*
 - C. botulinum*
 - C. acetobutylicum*
 - C. thermoaceticum*
43. Provide three products of *Bacillus* sporulation that makes it an important process from the industrial point of view:
- _____
 - _____
 - _____
44. Arrange the following in the order that they appear during sporulation:
- Cortex
 - Parasporal crystal
 - Dipicolinic acid
 - Assymetric spore septum formation
 - Spore coat
 - Heat resistant endospore release

Answer: _____

45. Why does *Clostridium acetobutylicum* make so much hydrogen gas?
- To remove acids from the growth medium so that the solventogenic portion of the fermentation may proceed.
 - To generate additional NADH for use in electron transport phosphorylation.
 - To generate additional NADH for making solvents.
 - To remove reducing equivalents (electrons) from the fermentation pathway so that acetyl-CoA and butyryl-CoA may be used to generate ATP.
 - To eliminate humans from the immediate environment.
46. Filamentous High Mol% G+C bacteria are usually....
- Soil organisms
 - Antibiotic producers
 - Gram positive
 - Gram negative
 - Rod-shaped
 - Fermentative
 - Found to use the Embden-Meyerhof-Parnas pathway of sugar metabolism
47. Which of the following is a genus:
- Actinomycetes
 - Streptomycetes
 - Streptomyces
 - Actinomyces
 - Actinobacteria
 - Actinoplanes
 - Nocardia
48. Mycolic acids are shared by one particular group of actinobacteria that includes the genera:
- Corynebacterium*
 - Arthrobacter*
 - Actinomyces*
 - Mycobacterium*
 - Nocardia*
 - Saccharopolyspora*
49. The compounds known as A-factor and geosmin are normally associated with:
- Pseudomonas* sp.
 - Bacillus* sp.
 - Ashbya* sp.
 - Streptomyces* sp.
 - Corynebacterium* sp.
50. Which of the following are typically found among the 'actinobacteria'?
- Endospores
 - Gram negative cell walls
 - High G+ C content
 - Nutritional versatility
 - Snapping division
 - Snapple division

51. Which of the following is true of the propionic acid fermentation carried out by *Propionibacterium*.

- a. One can find this type of fermentation in Swiss cheese and animal rumens.
- b. These organisms obtain ATP via the following reaction:
lactate \rightarrow pyruvate \rightarrow Acetyl-CoA \rightarrow Acetyl-phosphate \rightarrow Acetic acid
- c. Vitamin B₁₂-containing enzymes make methylmalonyl-CoA from succinyl-CoA.
- d. Propionic acid bacteria ferment the waste products of lactic acid bacteria.
- e. Lots of CO₂ and acetic acid are produced along with propionic acid.

52. Diphtheria is caused by _____. The toxin produced by this organism is unique in being encoded by genes on a _____. The toxin was the first example of an _____, a type of toxin first proposed to exist by Loeffler back in the 1880s.

53. Many bacteria grow on the end products of other bacteria present in the same environment. In the list below, identify a bacterial genus that must be present in the environment to supply the following organisms with essential sources of carbon and energy or appropriate conditions for growth.

Organism	Example Companion organism(s)
a. Propionic acid bacteria	
b. Methane oxidizers	
c. Methanogens	
d. Acetic acid bacteria	
e. OPRABs	

54. Which of the following statements is true concerning the *Archaea*:

- a. Some members of the *Archaea* have been identified only by 16S rDNA sequencing.
- b. The biochemistry of archaeal cell membranes and cell walls is very similar to members of the Bacteria.
- c. Archaeal enzymes involved in macromolecular synthesis (protein, DNA and RNA synthesis) are more similar to eukaryotic enzymes than bacterial enzymes.
- d. *Archaea* are famous for tolerating extremes of temperature, pH, salt and anaerobiosis.
- e. All *Archaea* are methanogens.

55. Provide three unique biochemical characteristics that are possessed by the *Archaea* but not by the *Bacteria* nor the *Eukarya*:

- 1.
- 2.
- 3.

56. Methanogens use a pathway for carbon assimilation similar to that used by:

- Methane-oxidizing bacteria
- Saccharomyces*
- Acetogenic (acetic acid producing) clostridia like *Clostridium thermoaceticum*
- Acetic acid bacteria like *Acetobacter*.
- Methylotrophs growing on formate.

57. Methanogens get energy and carbon from CO₂ and H₂. Upon examining the whole genome of *Methanococcus jannaschii* it became evident that it lacked the genes for phosphofructokinase, and the Entner-Doudoroff pathway. *M. jannaschii* did however have the genes for phosphoenolpyruvate synthase and other gluconeogenic enzymes. Based on what you know about metabolism of the methanogens, and how they assimilate their carbon, which of the following is the most likely route for anabolism in these organisms?

- Glucose ----> oxidative pentose phosphate pathway ---->TCA ----> PEP
- Formaldehyde ----> ribose 5-P pathway ----> Glucose
- Acetyl-CoA----> glyoxylate bypass ----> PEP ----> glucose
- Serine ----> Emden-Meyerhof ----> TCA ----> PEP
- Acetate ---> PEP ---> glucose ----> ribose 5-P pathway

58. Which of the following best describes the metabolism of methanogens:

- Electrons from methane oxidation are used to generate a proton motive force.
- Methanogens use unique cofactors to carry one carbon intermediates during methanogenesis.
- Methanogens can be considered autotrophs since they derive their carbon from CO₂.
- Methanogens get their energy via anaerobic respiration using electrons derived from H₂.
- Methanogens are obligately fermentative since they grow under strictly anaerobic conditions.

59. The following fermentation balance was achieved using an unknown bacterium growing on glucose.

Product	moles/100 moles glucose
Ethanol (CH ₃ CH ₂ OH)	198
CO ₂	199

The cell yield was calculated to be 10 grams per mole of glucose consumed.

- What pathway of glycolysis is being used (hint: remember $Y_{ATP} = g \text{ cells/mol ATP}$; a value of about 10.5 for any organism is usual)? _____
- What organism might this be? _____

60. Draw a phylogenetic tree for the following organisms:

Salmonella, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Agrobacterium*, *Bradyrhizobium*, *Streptomyces*, *Clostridium*, *Thermoplasma*.

61. Bacteriorhodopsin is produced by _____. The actual photopigment that undergoes a cis-trans conformational switch to generate a proton motive force is referred to as _____.
62. The observation that aeration suppresses fermentation has been called the _____. The observation that glucose represses respiration is referred to as the _____ effect. The latter effect is controlled in industrial fermentations by _____.

63. The following fermentation balance was achieved using an unknown bacterium growing on glucose.

<u>Product</u>	<u>moles/100 moles glucose</u>
Lactate (CH ₃ COHCOOH)	100
Ethanol (CH ₃ CH ₂ OH)	100
Acetate (CH ₃ COOH)	2
Succinate (HOOCCH ₂ CH ₂ COOH)	0
Formate (HCOOH)	0
CO ₂	100
H ₂	0

- a. What type of fermentation is this? _____
- b. Which product is the most oxidized? _____
- c. Which product is the most reduced? _____
64. Consider organisms growing on glucose as the sole source of carbon and energy. After anaerobic growth you find the following yields of cells per mole of glucose consumed:
- Organism A - 10 g
- Organism B - 24 g
- Organism C - No growth

Provide a genus name for each of the organisms that makes physiological sense (hint: remember Y_{ATP}).

65. Methanogens are important in biodegradation because they:
- Are nutritionally versatile and catabolize many carbon pollutants.
 - Tend to respire very rapidly and mineralize organic material to CO₂ and H₂O.
 - Grow at extremely high temperatures.
 - Consume hydrogen during the terminal degradation reactions.
 - Consume methane and pull the equilibrium of biodegradation towards completion.
66. Extreme halophiles, methanogens, and some thermophiles are typical of prokaryotes found in the:
- Euryarchaeota
 - Crenarchaeota
 - Archaea
 - Bacteria
 - Eukarya

67. Which of the following taxonomic groups of fungi has been made obsolete by modern methods in molecular phylogeny?
- Basidiomycetes
 - Ascomycetes
 - Zygomycetes
 - Deuteromycetes
68. Which of the following statements is true about Ascomycetes?
- Many Ascomycetes reproduce asexually by budding.
 - To be classified as an Ascomycete, an organism must reproduce sexually.
 - Ascomycetes make ascospores.
 - Many Ascomycetes form conidiospores.
 - Most Ascomycetes form sexual zygospores.
69. Most filamentous fungi are also:
- haploid
 - yeasts
 - coenocytic
 - anaerobes
 - aerobes
70. All fungi have the following in common:
- Numerous cross-walls in their hyphae.
 - Chitin in their cell walls.
 - Coenocytic hyphae.
 - Budding reproduction.
 - Ascospore production.
 - Nuclei.
71. The "Quick" process for manufacturing vinegar is an example of:
- An immobilized cell bioconversion
 - A large scale anaerobic bioconversion.
 - Using acetic acid bacteria for conducting partial oxidations.
 - Using petrochemicals to enhance the value of food.
 - A traditional process being converted to a large scale industrial process
72. In which of the following cases would isolated enzymes (either soluble or immobilized) most likely be used to carry out a process rather than whole cells?
- The reaction proceeds to equilibrium, for example: $X \rightleftharpoons Y$
 - The reaction required cofactors, for example: $X + \text{NADH} + \text{H}^+ \rightarrow \text{XH}_2 + \text{NAD}^+$
 - The reaction involves hydrolysis, for example: $\text{X-X-X-X} + \text{H}_2\text{O} \rightarrow \text{X} + \text{X} + \text{X} + \text{X}$
 - The reaction involves sequential conversions, for example: $\text{X} \rightarrow \text{Y} \rightarrow \text{Z}$
 - The reaction is subject to feedback inhibition in whole cells: $\text{X} \rightarrow \text{Y} \rightarrow \text{Z}$

73. During the "exponential" phase of growth of a non-filamentous bacterial culture, which of the following parameters give a straight line if plotted versus time on semi-log paper?
- Optical Density (OD)
 - Grams of protein
 - Grams of DNA
 - Dry weight of cells
 - Number of cells
74. Which of the following statements concerning the specific growth rate constant (μ) is true?
- μ remains constant regardless of the medium used, or growth temperature.
 - As μ increases, the generation time (g) also increases.
 - In some circumstances, mainly during exponential growth, μ can equal g .
 - μ changes rapidly just before and just after the exponential phase of growth.
 - μ is a constant that relates cell number to the rate of population growth.
75. In the Monod equation, the parameter K_s is:
- μ_{\max}
 - The substrate concentration where $\mu = \mu_{\max}$
 - The generation time.
 - How long the fermentation will run at a given substrate concentration.
 - The substrate concentration at which $\mu = 1/2 \mu_{\max}$.
76. In practice, which **one** of the following is adjusted to control the specific growth rate a chemostat?
- The dilution rate D .
 - The limiting nutrient concentration $[S]$ in the chemostat
 - The temperature
 - The generation time g
 - The flow rate F
77. Which of the following must be true to make generation times and specific growth rates useful for predicting the outcome of an industrial fermentation?
- The cell population must double at each generation time (i.e. 2, 4, 8, 16, 32,...).
 - The cell population must increase in an arithmetic fashion at each generation time (i.e. 1, 2, 3, 4, 5,)
 - The population must be in "balanced growth"
 - The environmental conditions (pH, medium, temperature) used must be the same for each fermenter run.
 - Mycelia must be formed by filamentous organisms.
78. Fed-batch processes are often used to overcome:
- The Crabtree Effect
 - The Glucose Effect
 - The Pasteur Effect
 - The toxicity of the substrate being used (for example acetate)
 - The toxicity of the product being formed (for example butanol)

79. In Type II fermentations, the product of interest is generally produced:
- When all growth has ceased.
 - During the most active period of growth.
 - When $\mu = \mu_{\max}$.
 - After the majority of growth has ceased
 - At a secondary stage of the fermentation.
80. If an organism has a "spontaneous mutation rate" of about 10^{-9} per gene per generation...
- The chances of a mutation occurring anywhere in the genome of a cell is about one in a billion
 - Each cell is guaranteed to have one mutation somewhere in its genome.
 - When an organism divides, there is a one in a billion chance of having a mutation in a given gene.
 - Plating 10^9 cells on an agar plate should yield one with a mutation in a particular gene.
 - If an organism has 1000 genes then, theoretically, one in one million organisms is a mutant of some kind.
81. Which of the following statements are true about 'strain improvement' programs?
- Most wild-type organisms need little improving to become production strains.
 - Mutational programs generally improve yields rapidly at first and then more slowly.
 - Best producers are usually obtained in a single mutational step; the cells are healthier than highly mutated strains.
 - Directed mutagenesis is best used for modifying complicated biochemical pathways (i.e. Vitamin B₁₂ production).
 - Mutants with altered morphological characteristics are generally not the best candidates for production strains.
 - The best production strain may not be the best strain that was identified by shake flask tests.
82. Which of the following occurs before a mutation occurs?
- DNA damage
 - Photolyase-mediated damage repair
 - Postreplicative recombination repair
 - Repair failure
 - DNA replication
83. A premutational structural change:
- Usually leads to a mutation
 - Is a mutation
 - Involves chemical modification of a nucleotide
 - Occurs with different frequencies depending on the mutagen used
 - Is usually repaired by DNA repair enzymes
84. Which of the following are not associated with ultraviolet light-induced mutagenesis?
- RecA
 - Thymine dimers
 - Photolyase
 - Alkylation
 - Base excision repair
85. Examples of negative regulation include.....
- A regulatory protein binding to DNA and blocking transcription.
 - A repressor protein releasing from DNA in the presence of an inducer.
 - The catabolite activator protein (CAP).
 - A corepressor binding to a repressor protein and then binding to DNA.
 - An mRNA leader sequence folding in the "terminator" conformation.

86. The role of LexA in the SOS response is to:
- Modify DNA polymerase so that it reads through gaps in complementary strands of DNA.
 - Act with RecA to promote cross-over in daughter-strand gap repair.
 - Act as a repressor of transcription for many DNA repair enzymes.
 - Act to turn off the SOS response once damage repair is complete.
 - Recognize mismatches caused by premutational structural changes.
87. Regulation of transcription by attenuation ...
- depends on how fast the ribosome translates the leader peptide.
 - can be called "posttranslational regulation".
 - can be referred to as "posttranscriptional regulation".
 - is mainly limited to amino acid biosynthetic operons.
88. The organisms isolated by Kinoshita that are now used for amino acid production are:
- Fungi
 - Biotin auxotrophs
 - Coryneform bacteria
 - Lactic acid bacteria
 - Filamentous prokaryotes
89. Analogue resistant mutants ...
- ...are usually auxotrophs.
 - ...may have an enzyme altered at an allosteric site.
 - ...have gained the ability to negatively regulate an operon.
 - ...grow in the presence of a normally toxic compound.
 - ...cannot grow in the presence of the compound the analogue replaces.
90. The usual way to select bacterial mutants that are auxotrophic for some essential metabolite is to carry out a:
- Random screening after mutagenesis.
 - Analogue selection after mutagenesis
 - Semisynthetic antimetabolite screening
 - Penicillin selection after mutagenesis
 - Protoplast fusion
91. Which of the following are reasons why wild-type *Corynebacterium glutamicum* overproduces glutamic acid?
- The organism lacks anaplerotic enzymes like pyruvate carboxylase.
 - The organism has low or undetectable levels of α -ketoglutarate dehydrogenase.
 - The organism cannot conduct glycolysis.
 - The organism requires biotin.
 - The organism is a glutamate auxotroph.
92. In contrast to MSG production, lysine production by *Corynebacterium glutamicum* can be characterized as:
- Due to rational selection of specific mutants.
 - More due to medium manipulation than to genetic improvement.
 - In part due to selection of analog resistant mutants.
 - In part due to selection of specific auxotrophs by penicillin enrichment.
 - Mainly due to their natural ability to overproduce most amino acids.

93. Large scale production methods for L-aspartate and L-alanine are good examples of:
- Batch culture techniques that operate without being subject to the glucose effect.
 - Immobilized enzyme technology.
 - Immobilized cell technology.
 - Continuous cultivation where $\mu < \mu_{\max}$.
 - Using cells for a single step bioconversion reaction.
94. Citric acid production is an example of...
- ...a Type I fermentation
 - ...a true bacterial fermentation.
 - ...the production of a primary metabolite.
 - ...production of a useful chemical from cellulose.
95. What do ascorbic acid, dihydroxyacetone and ampicillin have in common?
- All are made by partial oxidation of a reaction intermediate.
 - All are made partly by microorganisms and partly by chemical synthesis.
 - All are semisynthetic antibiotics.
 - All are made by *Gluconobacter*.
 - All are vitamins.
96. Which of the following are physiological reasons for the overproduction of citric acid?
- The thermodynamics of the TCA Cycle lead to citrate accumulation.
 - The producing organisms lack the glyoxylate pathway.
 - Producing organisms lack glycolytic pathways.
 - Ammonium ions relieve the inhibition of phosphofructokinase.
 - Iron is required for ferredoxin production.