

Microbe Mission B



November 16 - 24, 2024

Instructions:

- You will have **50 minutes** to complete your exam. At the end of this time period, you must stop working. Partial credit will be awarded, so attempt as many questions as you can!
- Each team is allowed **one** 8.5" x 11" note sheet with information on both sides which must be printed. If a sheet protector is used, it must be sealed with tape or laminated.
- Note that all **MCQs** have a “best” choice and do not have multiple answers. **Multiple-Select Questions** are designated with an asterisk at the beginning of the question, these will be scored with Scilympiad’s Formula. **Free Response Questions** will be scored in accordance with the scoring guidelines provided on the answer key.
- **Ties will be broken in accordance with the National Science Olympiad Rules in this order:**
 - ◆ **First: Section D (Molecular Biology) Score**
 - ◆ **Second: Section E (Metabolism & Applications) Score**
 - ◆ **Third: Section A (Microscopy) Score**

School/Team Name: _____

Team Number: B - _____

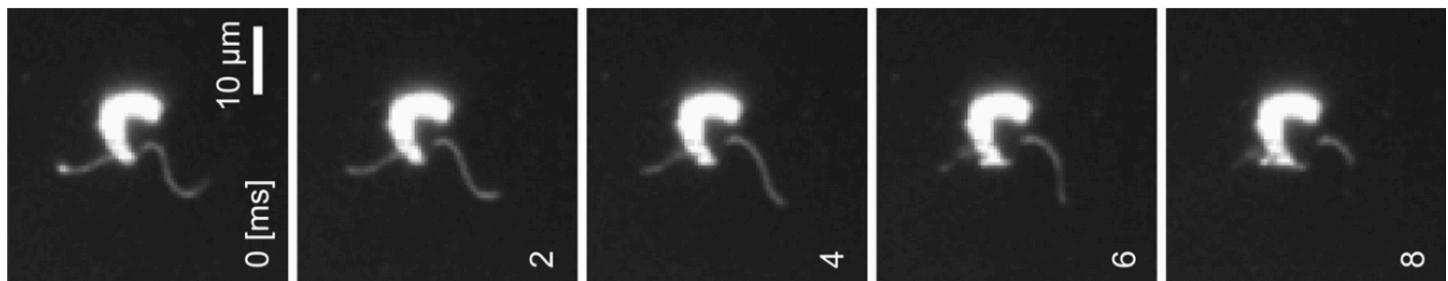
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Section A: Microscopy [27 pts]

These problems cover Section A of the Microbe Mission NSO Rules. Answer them to the best of your ability.

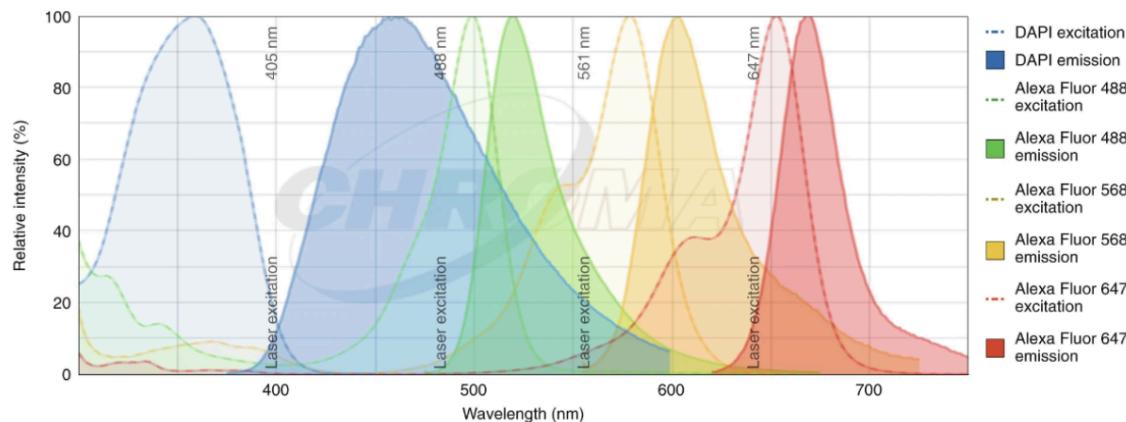
1. Which of the following statements about magnification is correct? Select all that apply. [2]
 - a. Microscopes are an example of a diffusion-limited system
 - b. Oil Immersion increases the numerical aperture of the microscope
 - c. The resolution limit of a microscope is equal to $0.5\lambda/n$ where n is the physical aperture and λ is the wavelength of light
 - d. You can resolve two closer points if they are blue than if they are red



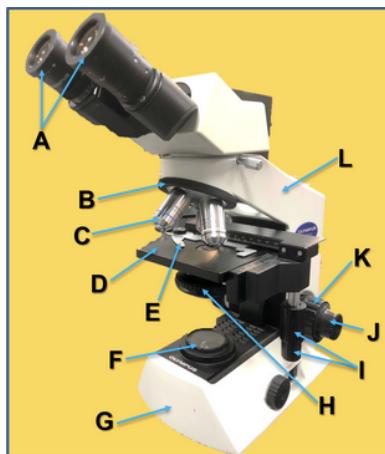
The image above shows the movement of an organism's flagella over time.

2. How do you know that this isn't fluorescence or electron microscopy. [1]
3. What special tool is used to achieve this type of microscopy? [2]
 - a. Phase Ring
 - b. Dichroic Mirror
 - c. Wollaston Prism
 - d. Condenser Lens
 - e. Direct Illumination Block
4. What could be a possible benefit or issue with this type of microscopy, specifically if you were imaging organisms that exhibited sensitivity to light? [2]

You begin your work at Dr. Parth's office. He tasks you with investigating a new series of Alexa fluorescent markers they are developing (Figure below)

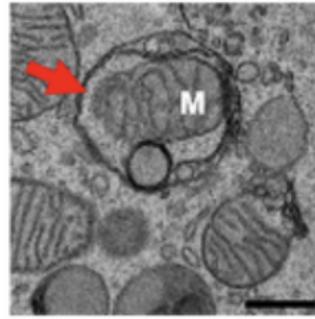
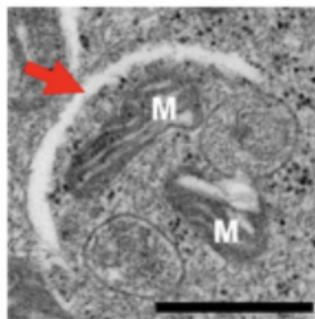


5. Of the four fluorescent markers available to you, which pair(s) would NOT be compatible for a FRET experiment? [2]
6. You have only a single laser, but you can set it to any wavelength of your choice. If you wish to visualize two different localities in different colors, what wavelength should you set it to? [1]
7. What organelle is DAPI most commonly used to visualize and why? [2]



8. Select all of the following that are true (use the above figure) [2]
 - a. The condenser is attached to H
 - b. The usual magnification on part A is 100x
 - c. A microscope should only be carried by L
 - d. I handles vertical translation of the stage
 - e. E is known as the stage claw
 - f. None of the Above
9. Scanning Electron Microscopy (SEM) would not be appropriate to observe which of the following [2]
 - a. Studying the motion of *Helicobacter pylori*
 - b. Looking at the sulfur bodies of purple sulfur bacteria
 - c. Visualizing the capsid of Tobacco Mosaic Virus

- d. Measuring the function of membrane bound receptors in *Giardia*
 - e. Cataloging extracellular receptors on the surface of *Saccharomyces cerevisiae*
10. Which of the following is a TRUE statement about the microscope image below? [2]



- a. These microscope pictures were taken using a scanning electron microscope (SEM)
 - b. The organelle represented by "M" in this image can use the glyoxylate cycle
 - c. The organelle represented by the red arrow will most likely transport the organelle represented by "M" to the vacuole
 - d. The process occurring in this image is common during fasting periods longer than 24 hours
 - e. If the process above was increased throughout the body, tumorigenesis would be more likely to occur.
11. Which of the following can you study in a bacteria with a scanning electron microscope (SEM)? [1]
- a. Nuclear Pores
 - b. Cell Membrane
 - c. Miniature Penguins
 - d. Fimbriae
 - e. Glycocalyx
12. Identify the tool in the below figure [1]



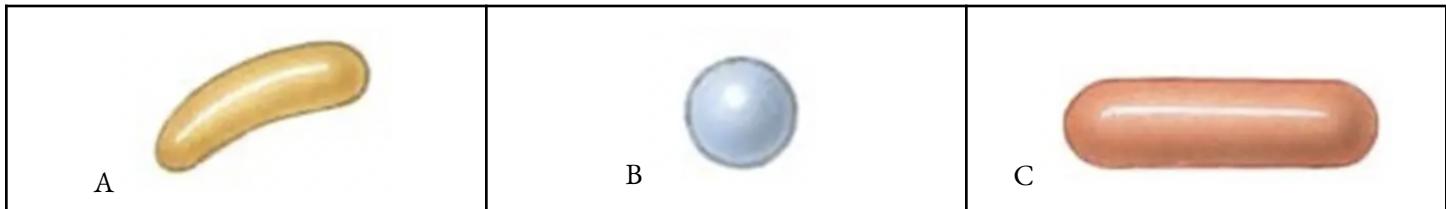
13. You have a saturated culture of yeast. You wish to perform a 1:1000 dilution using sorbitol. If you want to have a final volume of 1mL how much sorbitol do you need? Include units. [2]
14. Taking that diluted solution from question 13, put it on the device from question 12. If you see the image in the below figure, how many cells are shown in the image below that should be counted? [2]
15. What fraction of the total volume normally counted in the device does this represent? Write your answer as a fraction reduced to lowest terms. [1]
16. Let your answer to question 14 be x . Let your answer to question 15 by y . In terms of x and y , what is the concentration of cells in the original saturated solution from question 13? [2]

Section B: Structure/Morphology [30 pts]

Oh no! There has been some sort of outbreak at Mason High School in Mason, Ohio. You, as one of the world's most famous microbiologists, have been tasked to figure out what is the source of this outbreak and what agent is responsible. Below, you are given the names of the possible bacterial species involved, along with their gram stain result.

A	Bacillus Anthracis	+	E	Vibrio Cholerae	-
B	Staphylococcus Aureus	+	F	Streptococcus Pyogenes	+
C	Streptococcus Pneumoniae	+	G	Bacillus Masonis	-
D	Mycobacterium Tuberculosis	?	H	Diplococcus Validis	-

First, let's analyze the bacterial cell morphology for each of the following species above:



For the following questions, select all of the answer choices that apply.

1. Which bacteria (A-H) have the cell morphology shown in Image A? [1]
 2. Which bacteria (A-H) have the cell morphology shown in Image B? [1]
 3. Which bacteria (A-H) have the cell morphology shown in Image C? [1]
 4. One of your fellow microbiologists has isolated the mysterious bacterial strain and learned that it has a similar morphology as the bacteria pictured in the image below. Based on the SHAPE ALONE of the bacteria pictured below, which bacteria species (A-H) can be ruled out as a possibility for the mysterious bacterial strain in Mason, OH? [2]



Now that you have determined the shape of this mysterious bacteria, you still need more information in order to uncover its identity. You realize that the next best step to take is to perform a Gram Stain.

5. In order to begin performing your gram stain, you must first fix your mysterious bacteria to the slide in order to make sure that it does not get rinsed off during the procedure. This can be done by 2 main mechanisms: Chemical Fixation and Heat Fixation. Describe 1 advantage that Chemical Fixation has on Heat Fixation. [1]

6. What is the primary stain used in the Gram Staining procedure? What color are the gram negative cells after the addition of this primary stain? [2]

7. What property of crystal violet allows it to bind to the bacterial cell walls? [1]

8. What is the next reagent used in this procedure? [1]

9. After the addition of the reagent used in #XX, Ethyl Alcohol is added to the bacteria. What is the purpose of the addition of Ethyl Alcohol? What color will the Gram Negative cells appear? [2]

10. After applying your counterstain, Safranin, you look under the microscope to see the results of your perfect gram stain. You notice that none of the bacteria under the microscope appear pink. From the list of remaining possible bacteria, which bacteria can you determine is not the mystery bacteria? [1]

11. Which 2 bacteria are remaining? Briefly describe the differences in their cell wall composition and why they couldn't be easily distinguished by the Gram Stain. Additionally, state what staining procedure can be used to accurately reveal the identification of one of the remaining bacteria? [4]

12. Which of the following structures are common to both eukaryotic and prokaryotic cells? [1]

- a. Smooth Endoplasmic Reticulum
- b. Ribosomes
- c. Mitochondria
- d. Nucleoid Region

13. Which best describes the phospholipids in a bacteria that grows at lower temperatures? [1]

- a. Mostly Unsaturated
- b. Mostly Saturated
- c. Lacking polar phosphate groups
- d. Longer fatty acid chains

14. Which best describes the types of linkages found in the peptidoglycan cell wall of most gram positive bacteria? [1]

- a. Tetraglycine Cross-Linkages
- b. Tetraserine Cross-Linkages
- c. Pentaserine Cross-Linkages
- d. Pentaglycine Cross-Linkages

15. Which of the following structures would only be present in gram positive bacteria? [1]

- a. Lipoteichoic Acid
- b. Lipopolysaccharide
- c. Porins in outer membrane
- d. Pilus

16. According to the Baltimore Virus Classification System, which best describes the genome of a virus with Classification Group III? [1]

- a. dsDNA virus
- b. ssDNA virus
- c. dsRNA virus
- d. ssRNA virus

17. Which best explains how viruses attach to host cells in order to infect them? [1]

- a. Use surface proteins which bind to receptor molecules on the host cell membrane
- b. Synthesize messenger molecules telling the host cell to create a binding site
- c. Insert their genome onto the surface of the host cell creating an attachment site
- d. They do not need to attach because they originate inside of the host cell

18. List the steps of viral replication starting with Attachment [7]

Section C: Culture/Growth [35 pts]

1. You discover a cell line capable of undergoing all major metabolic pathways in animal cells. Four mutants of this cell line are known, with each mutant producing only one defective protein. You isolate each mutant (1-4) and grow them on a medium without glucose. Then, you add protein, carbohydrate, and fat solution to the media individually and measure the relative levels of ATP production by the cell. Protein solution consisted of various amino acids, carbohydrate solution consisted of various sugars, and fat solution consisted of triglycerides. In further experiments, you discover that when an inhibitor for beta oxidation is added to the animal cell, Mutant 2 no longer metabolizes fats. A “+” indicates high ATP production levels while a “-” indicates low ATP production levels. [5] Which of the following is TRUE about your experiments?
- On average, if proteins, carbohydrates, and fats were added to the media of each mutant, ATP production is highest in Mutant 1.
 - Fermentation occurs when protein is added to the media of Mutant 2 and Mutant 4.
 - Mutant 2 could produce defective phosphoglyceromutase enzymes.
 - Mutant 3 could produce defective pyruvate dehydrogenase enzymes.
 - Mutant 4 could produce defective aconitase enzymes
 - None of the Above

Mutant	Protein	Carbs	Fats
1	+	-	+
2	+	+	+
3	+	-	-
4	+	+	+

2. Which of the following describes a bacterial strain that would be blue in the presence of x-gal. The media is carbon restrictive but has lactose. [2]
- A wild type culture that has completely depleted all the lactose in the culture
 - A mutant with a mutant repressor that is incapable of binding DNA
 - A mutant with a mutant repressor that is incapable of binding allolactose
 - A mutant unable to convert lactose to allolactose
 - A merodiploid that has an extrachromosomal Lac operon with a constitutive promoter but no other genes
 - None of the Above
3. Which of the following describes a bacterial strain that would be blue in the presence of x-gal. The media is carbon rich with both glucose and lactose in abundance. [2]
- A wild type culture that has completely depleted all the glucose in the culture
 - A mutant with a mutant repressor that is incapable of binding DNA
 - A mutant with a constitutively active CAP
 - A mutant unable to convert glucose to any other hexose
 - A merodiploid that has an extrachromosomal Lac operon with a constitutive promoter but no other genes

-
- f. None of the Above
4. Select all of the following that are true about MacConkey Agar [2]
- The bile salts in MacConkey agar inhibits gram negative bacteria growth
 - It contains crystal violet, acid red, and lactose
 - MacConkey agar is commonly used to assess patients' skin microbiome
 - Helicobacter* colonies have mucoid forms on this medium
 - MacConkey agar is differential but not selective
 - None of the Above
5. Select all of the following that is NOT true about defined media [2]
- Defined media can include Bovine Serum Albumin
 - Defined media can never be complex
 - Defined Media can include Yeast Extract
 - Defined Media can be selective
 - Defined media can NOT be differential
 - None of the Above
6. Aseptic technique is not needed in which of the following scenarios [2]
- You are sequencing DNA from a pure culture of bacteria
 - You are replica plating *Saccharomyces cerevisiae*
 - You are mixing ingredients for media to go into the autoclave
 - You are streaking *E. coli* on a plate
 - You are performing a gram stain

Your friend Neil is trying to create mutant bacteria that can survive a mission to the moon. To do so, he enlists your help because of your expertise in microbe missions (and he's more of just an idea guy).

7. What is the specific serotype of the organism that is traditionally used in the Ames test? [1]
8. The organisms used in the Ames test are traditionally auxotrophs for what nutrient? [1]
9. The two most common tester strains used in the Ames test are known as T-1537 and T-1531. Which of the following statement(s) are true? Select all that apply [2]
- The Two strains show the same phenotype on SC-complete medium
 - The two strains show the same phenotype on minimal medium
 - T-1537 is a tester for indel mutations
 - T-1531 is a tester for indel mutations
10. What are the two Muller Morphs that could be best used to describe the situation where there's a loss of function mutation leading to auxotrophy? [2]

You review some preliminary work that Neil has done (poorly). He has done a fluctuation Ames test with the strong mutagen benzo[a]pyrene as a positive control (Row A) and epsom salt as the negative control (Row H) and no additives in Row G. Then in rows B-F he tested increasing concentration of a Mystery Compound. In columns 1-4 a tester strain for single nucleotide polymorphisms was tested. In Columns 5-8 a tester for frameshifts was tested. In Columns 9-12 a special strain (Strain X) was added that produces large amounts of bicarbonate (a buffer) as a respiratory product instead of CO₂ when unmutated but is not auxotrophic. The pH indicator in each well changes from purple to yellow when acid is introduced into the environment. All wells have media that does not contain histidine and was inoculated with the strains as indicated above. Assume all mutagenesis is 100% effective for controls. The figure below shows his results.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

11. An immediately obvious mistake is present in Row A. Benzo[a]pyrene is known to cause transversions which should revert the auxotrophy. What did Neil forget to add? What should the actual result of row A look like if Neil had added all the right additives? [4]

12. Row H is the negative control. What is this control telling you here with regards to your experimental lines? [2]

13. Neil is missing a sterility test in this setup. What is added to a well to conduct a sterility test? What is the purpose of a sterility test? [2]

14. What kind of mutations does the mystery mutagen cause? [2]

15. If you mixed Strain X and the frameshift tester, which of the following would you expect? Select all that apply [2].
 - This mixture would produce CO₂ rather than bicarbonate in respiration
 - A well with this mixture could be yellow in rows B-F
 - A well with this mixture in Row G would be purple

- d. This mixture could grow on minimal medium

Section D: Molecular Biology [21 pts]

1. Mr. Microbe is experimenting with the effect of mutations in a bacterial cell and the effect the mutations have on certain enzymes involved in DNA replication. He notices that one of the mutations he is studying causes the leading DNA strand to no longer be synthesized. Which enzyme was most likely affected by this mutation? [1]
 - a. DNA Polymerase I
 - b. DNA Polymerase II
 - c. DNA Polymerase III
 - d. DNA Polymerase IV

2. What is the most likely effect of a mutation in the DNA that produces the enzyme DNA primase? [1]
 - a. DnaA would not be able to bind to the OriC
 - b. DNA Polymerase would not be able to add new nucleotides
 - c. The lagging strand would dissociate from the replication fork
 - d. Replication would proceed as normal

3. What bond joins adjacent nucleotides together in a DNA strand? [1]
 - a. Phosphate Linkage
 - b. Phosphodiester Bond
 - c. Hydroxyl Bond
 - d. Glycosidic Linkage

4. Which of the following is not a component of the Origin of Replication in Bacteria? [1]
 - a. DnaA boxes
 - b. DNA unwinding element
 - c. Binding sites for initiation proteins
 - d. Holoenzymes

- *5. The DNA unwinding elements of the bacterial chromosome are mainly composed of which nitrogenous bases? **SELECT ALL THAT APPLY** [2]
 - a. Adenine
 - b. Cytosine
 - c. Guanine
 - d. Thymine

6. An important element of bacterial genome replication is the presence of SSBs. What does SSB stand for? What role do they play in replication alongside Topoisomerases? [3]

7. A bacterial genome with dsDNA is sequenced and found to contain 40% Adenine. What percent of Guanine can be found in this genome? [1]

- a. 10%
- b. 40%
- c. 50%
- d. Not enough information

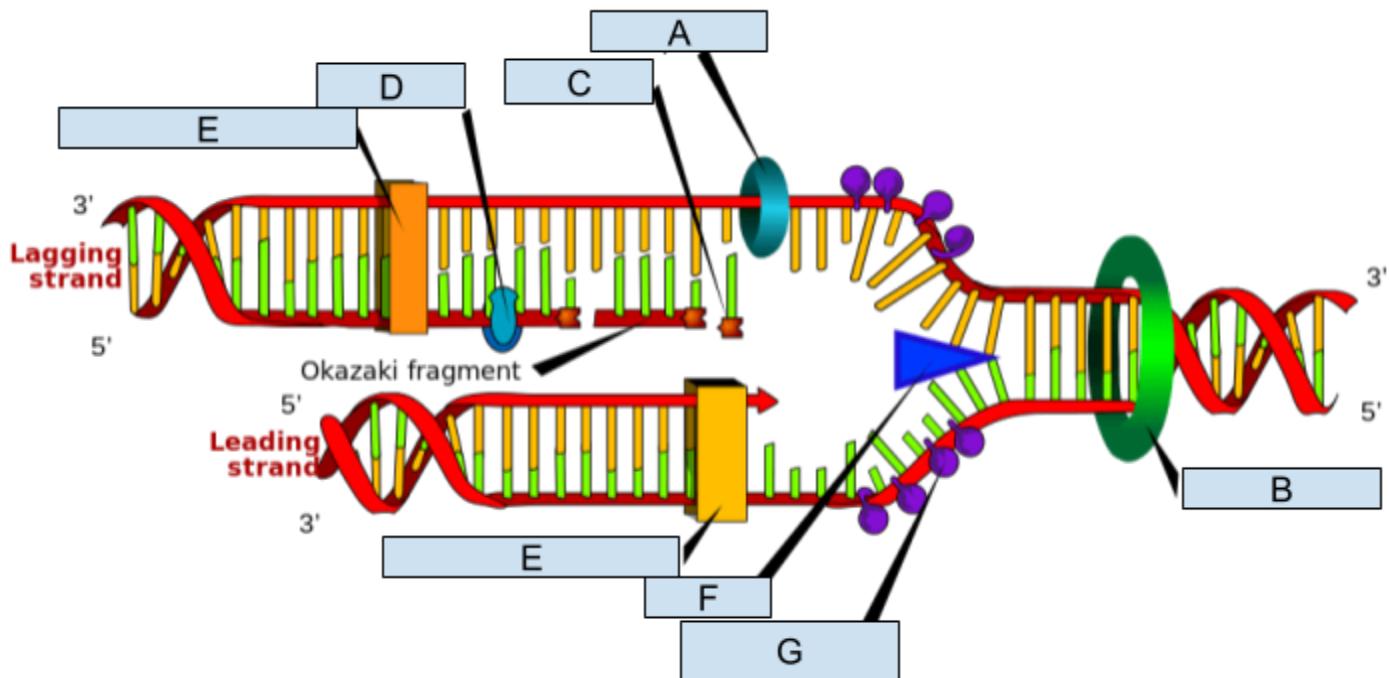
8. What is the name of the principle/rule used to answer the previous question? [1]

9. Now, a viral genome with ssRNA is sequenced and found to contain 40% Adenine. What percent of Guanine can be found in this genome? [1]

- a. 10%
- b. 40%
- c. 50%
- d. Not enough information

10. Explain the discrepancy in your answer to the previous question and the answer to #7. [2]

The following questions refer to the image below.



11. Identify Letter A [1]

12. Identify Letter B [1]

13. Identify Letter C [1]

14. Identify Letter D [1]

15. Identify Letter E [1]

16. Identify Letter F [1]

17. Identify Letter G [1]

Section E: Metabolism/Applications [18 pts]

You are studying the metabolism of a new species of microbes in outer space (how exciting). After obtaining these microbes, you expose them to many different conditions in order to determine and classify their metabolism type. After many years of research, you come to the conclusion that you are dealing with a Photolithoheterotroph. Answer the following questions.

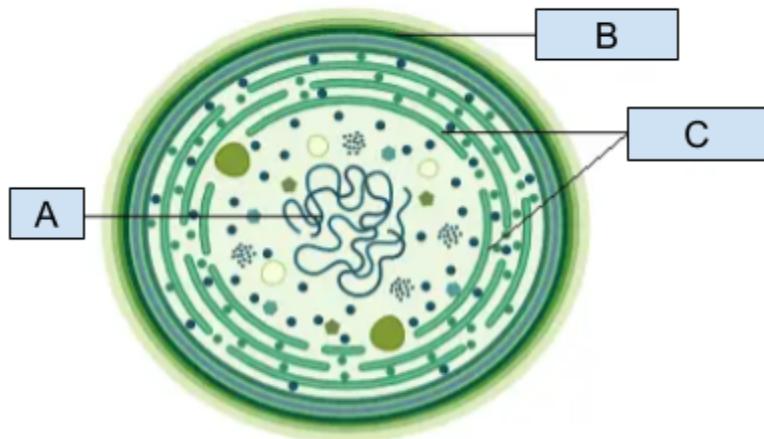
1. What is the energy source of this bacteria? [2]

2. Where does this bacteria get its source of electrons from? [2]

3. Will this organism take part in glycolysis? Answer yes or no [1]

4. If this organism does use glycolysis, where in this organism would glycolysis occur? [1]

Pictured below is a cyanobacterial cell. Use this diagram to answer the following questions.



5. Cyanobacteria are photosynthetic organisms. At which location (Give a Letter A-C) would photosynthesis take place in this organism? [1]

6. What is the name of the structure in the previous answer? [1]

7. Write the net reaction for photosynthesis. (Doesn't need to be balanced). [3]

8. What is the main type of fermentation used by microbes that contributes to bread rising? [1]

- a. Glycolytic Fermentation
- b. Lactic Acid Fermentation
- c. Aerobic Fermentation
- d. Ethanol Fermentation

9. What are the 2 main types of microbes (ex. Bacteria, archaea, etc.) involved in bread fermentation? [2]

10. During bread fermentation, microbes consume sugars and produce gases as byproducts. The production of which gas is the main cause of the actual rise of bread during baking? [1]

- a. O₂
- b. CO₂
- c. H₂O (g)
- d. CH₃COOH (g)

11. *Nannochloropsis*, *Chlorella*, and *Dunaliella* are all types of which microbe commonly used in biofuels? [1]

12. What process in the organisms listed above allows them to fix CO₂ into carbohydrates and lipids? [1]

13. Under stress (such as nitrogen starvation), many of these species shift toward storing energy as lipids and oil. What is the important role that these lipids and oils can play in industry? [1]

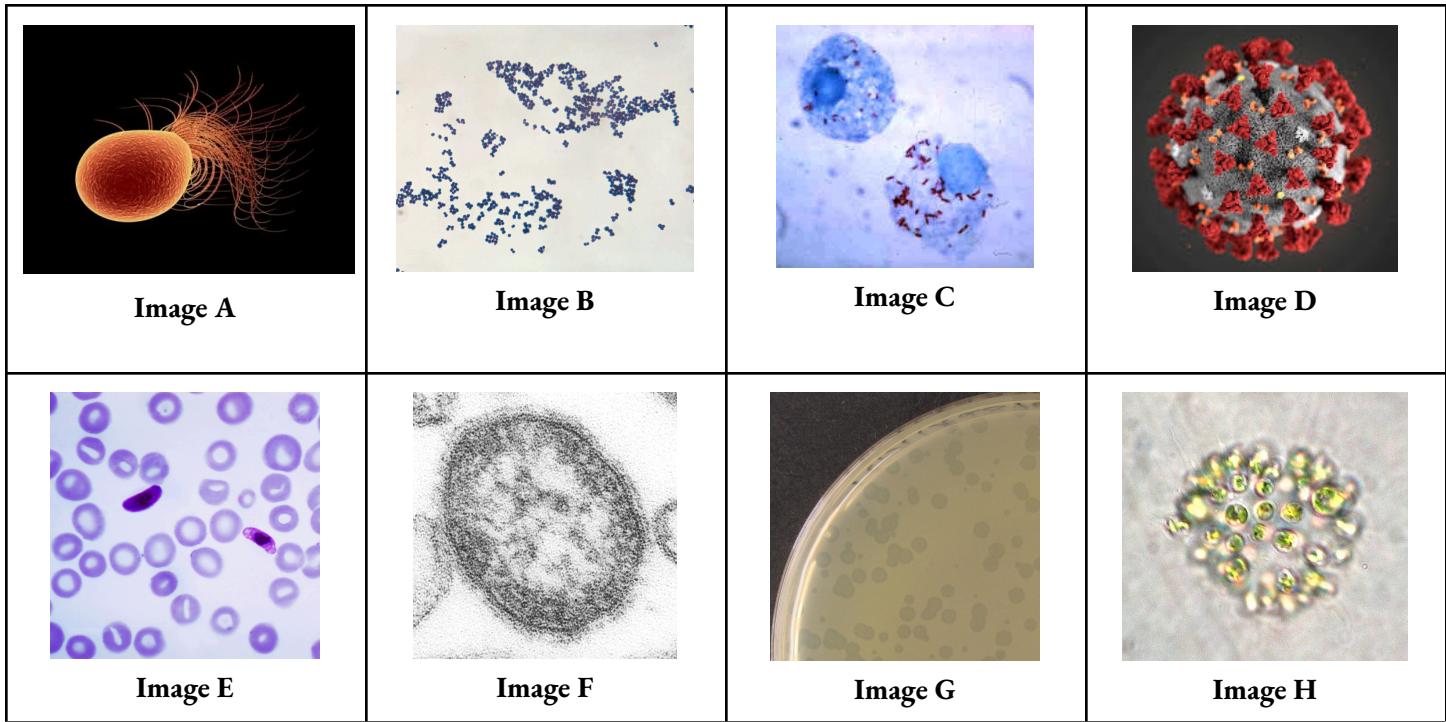
Section F: Evolution/Ecology [## pts]

1. Match the extremophiles to their descriptions. 0.5 pts awarded for each match. [3]

a. alkaliphile	_____ lives underneath rocks in cold deserts
b. capnophile	_____ optimal growth at a pH greater than 9.0
c. halophile	_____ lives in microscopic spaces within rocks
d. cryptoendolith	_____ grows in high concentration of CO ₂
e. Psychrophile	_____ optimal growth below 15 C/59 F
f. hypolith	_____ grows at a concentration of dissolved salts greater than 50 g/L
2. Which method of horizontal gene transfer is used during the replication of lytic and temperate bacteriophages? [1]
 - a. sexual reproduction
 - b. conjugation
 - c. transduction
 - d. Transformation
3. Which of the following is NOT a limitation of 16S rRNA gene amplicon sequencing? [1]
 - a. the relative abundance of all bacteria in the sample cannot be determined
 - b. primers used for amplification will introduce a bias as they bind to regions that aren't 100% conserved across all bacteria
 - c. bacteria can only be identified to genus level due to high similarity between 16S rRNA gene
 - d. does not provide information on antibiotic susceptibility
4. Which of the following relationships is an example of syntrophyism? [1]
 - a. plasmodium bacteria living within and exploiting vertebrates
 - b. lactic acid bacteria inhibiting candida albicans
 - c. fungi and algae combining to produce algae with the necessary characteristics to survive
 - d. dust mites living off of human skin flakes
5. State the four pieces of evidence supporting the validity of the endosymbiotic theory. [4]

Section G: Microbes and Agents [34 pts]

Identify and answer questions about the following microbes from the 2024-2025 Microbe Mission Microbes and Agents List.



1. Identify the Microbe in Image A. [1]

2. What type of microbe is this? [1]

3. This microbe is known to be a thermophile. What is a thermophile? [2]

4. Identify the Microbe in Image B. [1]

5. What type of microbe is this? [1]

6. This organism tests positive for a common diagnostic test known as the catalase test. What reagent is added to this organism in the catalase test? What would be observed in a positive catalase test when used on this microbe in the lab? [3]

7. Identify the Microbe in Image C. [1]

8. What is the name of the disease that this microbe causes? [1]

9. What are the vectors that are responsible for the transmission of this disease? [1]

10. Identify the Microbe in Image D. [1]

11. According to the Baltimore Virus Classification System, what type of virus is this? [1]

12. What important enzyme is carried by viruses of this classification and what is the function of this enzyme? [3]

13. Identify the Microbe in Image E. [1]

14. What is the name of the disease that this microbe causes? [1]

15. What organ in the human body does this microbe replicate in during infection? [2]

16. In Image E, what type of stain was used to visualize this microbe? [1]

17. Identify the Microbe in Image F. [1]

18. What type of genome does it have? [2]

19. What is the site of infection of a person infected with this microbe? [2]

20. Identify the Microbe in Image G. [1]

21. In Image G, this microbe is being cultured on an agar plate. What are the dots of varying size on the agar plate called? [1]

22. Identify the Microbe in Image H. [1]

23. This microbe is known to produce many toxins. Name one of the toxins produced by this microbe. [1]

24. This microbe is also known to contribute to many HABs. What are HABs and what water conditions usually lead to formation of HABs? [3]