1. A major practical problem for microbiologists is isolation of a specific microbe when microorganisms are present in very low numbers in a sample. Design and explain your experimental procedure for the isolation of bacteria that degrade the herbicide 2,4-dicholorophenoxy acetic acid (2,4-D) (6 points)

Inoculation in liquid media containing **2,4-D as its sole carbon source (or nutrient etc.) (4 points)** and the required nitrogen, phosphorus, sulfur, and mineral components. After incubation a sample of the culture is transferred to a **selective medium (2 points)** for further **enrichment media (2 points)**. Plating the sample on agar containing 2,4-D. Obtain the pure bacteria

(**keywords in the bold face should be appeared**)

2. What advantage does a microbe gain by using active transport rather than facilitated diffusion? (4 points)

Microbes live in **the environment having low concentration of nutrients**. They have to uptake the nutrients against the concentration gradient. Active transport is transport of solute molecules to higher concentrations with the input of metabolic energy for efficiently moving molecules into the interior. Facilitated diffusion depends on the concentration.

3. Why is RNA thought to be the first self-replicating biomolecule? (4 points)

RNA was capable of **storing, copying, and expressing genetic information**, as well as catalyzing other chemical reactions (e.g**. ribozyme**) and used as metabolic currency (e.g. **ATP**).

4. What is the difference between a microbial species and a strain? (4 points)

Microbial species are a collection of strains that share many stable properties

Strain is the descendant of a single, pure microbial culture.

5. All four nucleic acid forms can serve as viral genomes. Describe each (4 points).

ssDNA: ΦX174 and M13
dsDNA: most bacterial viruses
ssRNA: most plant viruses
dsRNA: infect animals, plants, fungi, and at least one bacterial species(e.g. Φ6 and rotaviruses)

6. How does resolution depend on the wavelength of light, refractive index, and numerical aperture? (3 points)

$$d = \frac{0.5\lambda}{nsin\theta}$$

d=resolution, λ=wavelength of light, *n*=refractive index, *nsinθ*=numerical aperture

7. Describe the Gram-staining procedure and explain what happens to a bacterial cell at each step. What step in the procedure could be omitted without losing the ability to distinguish between gram-(+) and gram-(-) bacteria? Why? (7 points)

   1. Sample preparation (heat fixation)
   2. First Staining with crystal violet dye – cell wall stained with crystal violet dye
   3. Mordanting with iodine – dye will be strongly bound with cell wall
   4. Decolorization with ethanol – dye will be removed from cell wall depending on the thickness of cell wall
   5. Counterstaining with safranine

Step 5 can be omitted. In step 4, **gram(+) (purple) and gram(-) (colorless)** cells can be recognized.

8. What is the relevance of the surface area-to-volume ratio in the size and shape of microorganisms? (4 points)

Being small increases the surface-volume ratio (S/V ratio). As this ratio increases, **nutrient uptake and diffusion of molecule within the cell become more efficient, increasing growth rate.** Small size is more beneficial.

Shape affects the S/V ratio. **A rod shape has higher S/V ratio than a coccus.**

9. Why does peptidoglycan contain the unusual D-isomers of alanine and glutamic acid rather than the L-isomers observed in proteins? (4 points)

Peptidase degrade L-amino acid. So, the presence of D-amino acids protects against degradation by those peptidase.

10. What do you think would be the outcome if you were able to 'transplant' CreS into a rod-shaped bacterium such as *Bacillus subtilis*? (4 points)

Induces curvature in rods

11. Compare the membranes of *Eukarya*, *Bacteria* and *Archaea*. (6 points)

Eukarya : double layered, lipid having ester bond, including sterol such as cholestenol

Bacteria : double layered, lipid having ester bond, including hopanoids

Archaea : double or mono layered composed of diether or tetraether, lipid having ether bond,

12. What is the difference between chromatin and chromosomes? (4 points)

Chromatin is a complex of DNA and protein as histones

Chromosome : condensed chromatin

13. What features of mitochondria support the endosymbiotic hypothesis of their evolution? (4 points)

Similarity to the shape and size of bacteria

Closed circular DNA

Ribosomes are the same size to bacteria

16s rRNA sequences are most similar to bacteria

Binary fission

14. Define yourself in terms of nutritional types (e.g. requirements and sources of carbon, energy and electrons) (4 points)

Heterotroph (C source), Chemotroph (Energy), Organotroph (Electron) or Chemo- Organo- Hetero- troph

Define or describe following terms (2 points each)

15. Pleomorphic

16. LUCA **(full name)**

17. The germ theory of disease

18. Hopanoids

19. Lysogeny

20. Temperate phage

1) Louis Pasteur , 2) Shibasaburo Kitasato, 3) Elie Metchnikoff, 4) von Behring, 5) Edward Jenner

21. Prophage

32. Settling spontaneous generation controversy

22. Curing

1) Louis Pasteur, 2) John Tyndall, 3) Ferdinand Cohn

23. Episome

24. Lysosome

John Needham, Joseph Lister, Antony van Leeuwenhoek, Theodore Schwann, Carl Woese, Edward Jenner, John Tyndall, Ferdinand Cohn, Richard Petri, Shibasaburo Kitasato, Robert Koch, Robert Hooke, Louis Pasteur, Elie Metchnikoff, von Behring, Dimitri Ivanowski, Martinus Beijerinck, Lazzaro Spallanzani, Felix Pouchet

25. Phagocytosis

26. CSLM **(full name)**

27. SEM **(full name)**

28. LPSs **(full name)**

29. PHB **(full name)**

Pick scientists related to following subjects from the list (1 point per scientists; a wrong answer will make a minus point)

30. Microbial ecology

1) Martinus Beijerinck

2) 'Sergei Winogradsky' will be 2 points though he is not in the list)

31. Immunology