Please, answer to all the following five questions and be concise (i.e., brief but comprehensive). Be sure to check carefully what is asked, and define all of the terms you use and use them logically in your answers.

1. A study of the human gut performed in 1982 used various types of plates under different growth conditions to describe and enumerate the microbes in the human gut. They concluded that the majority of the microbes in the human gut are Gammaproteobacteria (a class of bacteria in the phylum Pseudomonadota). A study conducted in 2010 using next-generation sequencing to characterize 16S rRNA sequences concluded that the majority of the microbes in the human gut belong to the phyla of Firmicutes and Bacteriodetes. Why did these studies have different conclusions? Which study do you think is most accurate?

   Max 10 points

2. On 24 February 1988, evolutionary biologist Richard Lenski filled 12 flasks with sugary growth medium and inoculated each with *Escherichia coli* bacteria at Michigan State University in East Lansing. Since then, the bacterial cultures have been nurtured, refreshing growth media daily and freezing samples for future study every couple of months. Are changes in the genotypes or phenotypes of the bacterial populations to be expected in such an experiment? Why/why not? Why do you think it was relevant to start as many as 12 independent cultures?

   Max 10 points

3. Aromatic compounds in the lignin hydrolysates are generally toxic to microorganisms but *Rhodococcus opacus* has been found able to tolerate and metabolize the aromatics. However, different strains of the same species may vary in the tolerance and capability to metabolize the compounds. Propose an experiment to rank a set of *Rhodococcus opacus* strains based on their specific growth rate on a lignin hydrolysate. How could the specific growth rates of the strains be determined (i.e, explain what is the data needed, how and when should it be collected, and how is it analyzed to determine the specific growth rates)? Are there other relevant growth parameters to consider in order to evaluate the performance of the strains? Give an example of how to determine a selected one.

   Max 15 points

4. All microbes need to conserve energy, have reducing power, and achieve redox balance. They fulfill these needs with diverse strategies. Explain briefly what these needs mean, and give an example of the diverse ways each of these fundamental needs can be fulfilled by microbes, **or** assess and report, based on the text below (adopted from Wilkening et al. Biochimica et Biophysica Acta (BBA) – Bioenergetics (2019) 1860:148062), how *Cupriavidus necator* fulfills the fundamental needs.

   Cupriavidus necator (formerly *Ralstonia eutropha*) is a gram-negative β-proteobacterium endowed with a versatile and adaptive metabolism. The bacterium is able to oxidize organic compounds, preferably organic acids, but is also capable of exploiting CO_2 and molecular

hydrogen (H_2) as carbon and energy sources, respectively [1]. The ability to metabolize H_2 is mediated by four distinct [NiFe] hydrogenases in *R. eutropha* [2] that catalyze the (reversible) oxidation of molecular hydrogen in the presence of oxygen (O_2) [3]. Two of these O_2-tolerant hydrogenases are directly involved in energy conversion processes of the bacterium. The membrane-bound hydrogenase (MBH) oxidizes molecular hydrogen and channels the released electrons via a membrane-linked cytochrome b into the respiratory chain [4]. The second hydrogenase is the cytoplasmic soluble hydrogenase (SH), which couples H_2 oxidation to the reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) to NADH [5]. NADH can then be used as a reducing agent for, e.g., CO_2 fixation via the Calvin-Benson-Bassham cycle or for ATP generation via the respiratory chain [1].

Max 10 points

5. Two common levels of regulation of protein activity are transcriptional control and posttranslational control. Describe examples of the two types of regulation and discuss the differences between the two in terms of speed of response in the phenotype or the function of the cells and energy efficiency.

Max 10 points