Final Exam: In-class questions | MB 451 Microbial Diversity

Honor pledge: “I have neither given nor received unauthorized aid on this test.”

Name: ____________________________________________ Date: __________________________

4. What are the 3 primary branches of life? (5 points)

Multiple-choice questions (1 point each)

5. ____ How did Reysenbach (and co-workers) confirm that the sequence EM17 really was the pink filamentous organism?
   A. Denaturing gradient gel electrophoresis
   B. Fluorescent in situ hybridization
   C. Terminal restriction fragment length polymorphism
   D. Real-time polymerase chain reaction
   E. All of the above

6. ____ In Denaturing Gradient Gel Electrophoresis (DGGE), DNA molecules are separated by...
   A. G+C content
   B. Denaturation point
   C. Length
   D. Sequence complexity
   E. None of the above

7. ____ The carbon-fixing symbionts in the scaly snail reside...
   A. In their gills
   B. In the surface layer of their scales
   C. In their liver and spleen
   D. In their muscular foot
   E. In their esophageal glands

8. ____ Which of the following is not one of the four phyla most abundant in/on the human body?
   A. Bacteroids
   B. Proteobacteria
   C. Firmicutes
   D. Cyanobacteria
   E. Actinobacteria

9. ____ Which of the following human microbiomes cannot be distinguished?
   A. Male from female
   B. Dominant hand from non-dominant hand
   C. Oral cavity from gut
   D. One individual from another
   E. All of these can be distinguished

10. ____ If you want to determine in a Stable Isotope Probing (SIP) experiment who in an environment is fixing nitrogen, the stable-isotope probe would be ...
    A. $^{13}$CO$_2$
    B. A $^{32}$P-labeled rRNA primer
    C. $^{15}$N$_2$
    D. $^{133}$Cs-tetrafluoroacetate
    E. None of the above
11. ____ What approach did Yim (and co-workers) use to examine the microbial communities from the Tibetan hot spring?
   A. Denaturing gradient gel electrophoresis
   B. Fluorescent in situ hybridization
   C. terminal restriction fragment length polymorphism
   D. real-time polymerase chain reaction
   E. all of the above

12. ____ Proteorhodopsin is a ...
   A. sensory opsin
   B. light-driven chloride pump
   C. light-driven proton pump
   D. light-detecting calcium channel
   E. pseudogene

13. ____ Which of the following unicellular eukaryotes (protists) was found by Moreno (and co-workers) to preferentially feed on autotrophic ammonia-oxidizing microbes in wastewater sludge?
   A. Arcella
   B. Chaos
   C. Epistylus
   D. Spumella
   E. Hartmanella

14. ____ What was the primary method used by Sakamoto (and co-workers) to compare oral microbial populations before and after periodontal treatment?
   A. FISH
   B. DGGE
   C. tRFLP
   D. rtPCR
   E. SIP

Short-answer questions (5 points each)

15. What is “horizontal gene transfer” and list 3 ways horizontally transferred genes be detected.
16. Describe in detail the most interesting thing you learned from any of the papers we reviewed in class. Make sure to include why you think this is interesting - what does it mean to you? This is a substantial question - please give a substantial answer.
17. Describe **one** of the following technologies: stable-isotope probing (SIP), terminal RFLP (tRFLP), or denaturing gradient gel electrophoresis (DGGE).
Essay question (10 points)

18. Describe in detail any paper we’ve discussed in this course. You are allowed to use papers we reviewed in the Discussion session if you wish.
Papers reviewed in class:

- Moreno Am, Matz C, Kjelleberg S & Manefield M 2010 Identification of ciliate grazers of autotrophic Bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing. AEM 76:2203-2211

Papers reviewed in the Discussion sessions this semester (you can use these if you wish):

- Rault D, et al. 2008 Nanobacteria are mineralo fetuin complexes. PLOS Pathogens 4:e41
- RASMUSSEN ET AL., EARLY DIVERGENT STRAINS OF YERSINA PESTIS IN EURASIA 5,000 YEARS AGO. CELL 2015 163:571-582
1. Imagine you have woodlice (also known as sow bugs, pill bugs, roly-polys) in Microbial Diversity lab. Droppings from these bugs examined under a microscope reveal large numbers of filamentous organisms that look like *Bacillus* (not *Clostridium*), complete with endospores. When you plate bug droppings out on PYD and incubate aerobically, you get lots of identical-looking *Bacillus* colonies, but the cells are typical individual cells, or at most pairs. The ssu-rRNA sequences from these colonies are a perfect match to *B. cereus*, a common soil *Bacillus* species. Your TA tells you to keep trying, looking for a different colony type, until you get the filamentous *Bacillus* to grow on plates. But it doesn’t seem to work, you keep getting *B. cereus* and random non-*Bacillus* things, no filamentous organisms. You think that the filamentous *Bacillus* might not be able to grow on the media you’re using, and so the only thing you get on plates is easy-to-grow *B. cereus* that’s also in the droppings. But Dr. Brown suggests that *B. cereus* might be pleomorphic; that it might grow as filaments in bug intestines (and so in the droppings) but as individual cells in culture; in other words that the individual-celled *B. cereus* you’re growing might actually be the the same thing as the filamentous *Bacillus* in the bug droppings. You decide to figure it out once and for all in a research lab the following semester. How would you go about this? What would the results look like either way? Are there other alternatives? Be sure to provide important experimental details. (20 points)

2. Imagine you’ve discovered a novel species of fish in a deep-sea methane (CH$_4$) seep. This fish completely lacks a digestive tract, including both gullet and anus - the mouth opens to the gills but no further. However, it gets along just fine, swimming around in methane-infused water. You hypothesize that it’s absorbing both methane and oxygen from the water with its gills, and is living by methane oxidation (CH$_4$ + O$_2$ -> CO$_2$ + H$_2$O) rather than eating. Recognizing that the fish also needs an organic nitrogen source (which would normally be acquired in the diet), you further hypothesize that the fish is somehow getting nitrogen from the N$_2$ also present in significant amounts in the seep environment. You dissect one of these fish, and discover a grossly enlarged liver filling the space where the GI tract normally would be. Microscopic examination of the cells of this organ show both spherical and rod-shaped bacterial endosymbionts, and a rod-shaped bacterial symbiont in the interstitial spaces. (1) How would you identify these apparent symbionts, and (2) how would you determine which (if any) of these are carrying out methane oxidation and/or fixing nitrogen? There is no need to describe how the general techniques involved work, but be sure to provide the specifics required to make these techniques provide the answers you’re looking for. Note that neither the bacteria nor the fish can live in the lab, and so stable-isotope probing (SIP) is not an option. Be sure to tell me exactly what you’re looking for in your experiments - be specific. (20 points)

3. In the paper “Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes” by Sakamoto, et al, the authors use t-RFLP, with some help from realtime PCR and traditional ssu-rRNA clone-and-sequence approaches, to see how periodontal microflora in periodontal disease patients changed after treatment. This paper is now more than 10 years old. How would you do this experiment today with modern techniques? (20 points)
Don't Panic!