
Final Exam : Take-home questions | MB 451 Microbial Diversity

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

The Rules : You are allowed to use any book or online resources to help answer these questions. You are *not* allowed to talk with or work with anyone else (whether they are students in this course or not) about these questions.

Signed : _____

Date : _____

Name : _____

1. Summarize this paper (10 points):

Reysenbach AL, Wickham GS & Pace NR 1994 *Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park*. Appl. Env. Microbiol. 60:2133-2199

... with respect to the following points:

- A) What question or problem does this paper address? In other words, what was the purpose of doing this work?
- B) What system do they work with? Think field sites, organisms, locations, this sort of thing.
- C) What approaches did the authors use? Don't recite the Materials & Methods, just outline the methods and how they might have fit together, and any crucial details such as primers, probes, &c.
- D) What were the main conclusion(s) of the paper?
- E) What evidence did they present to support these conclusions?

2. Summarize this paper (10 points):

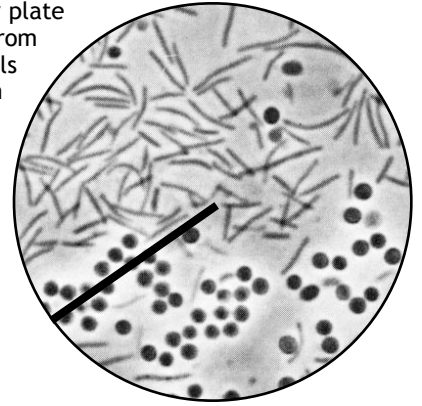
Moreno Am, Matz C, Kjelleberg S & Manefield M 2010 *Identification of ciliate grazers of autotrophic Bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing*. AEM 76:2203-2211

... with the following points:

- A) What question or problem does this paper address? In other words, what was the purpose of doing this work?
- B) What system do they work with? Think field sites, organisms, locations, this sort of thing.
- C) What approaches did the authors use? Don't recite the Materials & Methods, just outline the methods and how they might have fit together, and any crucial details such as primers, probes, &c.
- D) What were the main conclusion(s) of the paper?
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3. In MB 452 Microbial Diversity lab, imagine you have isolated colonies on your agar degrader plate that contain both long rods and large cocci. No matter how many times you re-streak from well-isolated single colonies, you get the same mixture of cell morphologies. Your TA tells you to keep trying until you get the two different organisms separated, but it doesn't seem to work and it occurs to you that they could be symbionts, two species can't grow separately. On the other hand, Dr. Brown tells you it might be a single pleomorphic species, that the cocci are stationary-phase cells and the rods are growing cells.

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? Be sure to provide important particulars such as probes, labels, primers, &c. (10 points)



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4. "*Chlorochromatium aggregatum*" is not a single organism, but consortia of two symbiotic organisms. Each of the colorless rod-shaped bacteria is surrounded by about 20 green cells. The rRNA sequences of both kinds of organisms have been determined; the colorless central rod-shaped cells are beta-proteobacteria that are not close relatives of any cultivated organism. The green surrounding cells are species of *Chlorobium*. These consortia cannot be grown in pure culture, but they predominate the microbial communities at the chemocline of meromictic lakes, and can be highly enriched from these samples by repeatedly shining a light on a sample and collecting the organisms that swim to the light. Any enriched sample of "*Chlorochromatium aggregatum*" contains what look like several kinds of related sets of organisms, and it has been confirmed in the phylogenetic analyses that not all consortia contain the same kinds of Chlorobi or beta-proteobacteria.

It is presumed that the *Chlorobium* is fixing carbon for consortia, since all known Chlorobi are obligate photoautotrophs. But what about nitrogen? These consortia can grow nicely with only atmospheric nitrogen present (no measurable nitrite, nitrate, ammonium or organic nitrogen) and so must fix nitrogen. How would you go about determining which of these two kinds of organisms fixes nitrogen for the consortia? How would the fact that you'd be isolating a mixture of related consortia (not a "pure" consortium) impact your results? (10 points)

NOTE : Stable-isotope probing won't work in this experiment. For 5 extra credit points, explain why not.

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5. 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 primarily 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 7 years old, and the experiments were probably designed about 10 years ago. How would you do this experiment today? What would be the advantages of the more modern approach? (10 points).

USE THIS PAGE TO FINISH ANY ANSWERS YOU'RE BEING LONG-WINDED ABOUT

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

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

Multiple-choice questions (2 points each)

7. ____ The pink filaments of Yellowstone National park have been cultivated and named...

- A. *Thermus aquaticus*
- B. *Thermoleophilum album*
- C. *Thermophilum pendens*
- D. *Thermocrinus ruber*
- E. none of the above

8. ____ The carbon-fixing symbionts in the scallop reside...

- A. in the esophageal gland
- B. in the muscular foot
- C. on the scales
- D. in the gills
- E. none of the above

9. ____ 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

10. ____ "Unifrac" is ...

- A. a measure of the similarity of microbial populations
- B. a 3D plot of principle-component analysis
- C. a tree-generation algorithm
- D. a giant computer from the 1960's
- E. none of the above

11. ____ A "phylogenetic probe" is ...

- A. A fluorescently-labeled oligonucleotide complementary to a region of rRNA used in FISH experiments
- B. a heavy-isotope-labeled growth substrate used to label rRNA in SIP experiments
- C. an ion-specific probe coated in specific oligonucleotides that can measure organisms *in situ*
- D. a program that scans metagenomic sequences for phylogenetic signal to identify the source organism
- E. none of the above

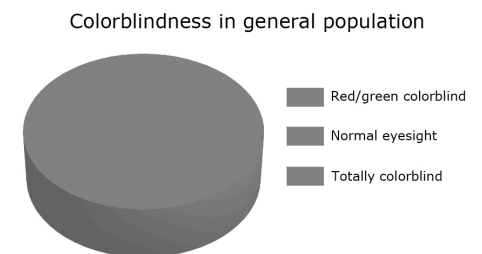
12. ____ The biggest advantage of using terminal RFLP to identify organisms in an environment is ...

- A. it's well-established and reliable
- B. it's quantitative
- C. it avoids PCR and the associated artifacts
- D. you don't have to sequence anything
- E. none of the above

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13. ____ 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. $^{13}\text{CO}_2$
 - B. a ^{32}P -labeled rRNA primer
 - C. $^{15}\text{N}_2$
 - D. ^{133}Cs -tetrafluoroacetate
 - E. none of the above
14. ____ Proteorhodopsin is a light-driven ...
- A. Cl^- pump
 - B. H^+ pump
 - C. Ca^{++} channel
 - D. ATPase
 - E. none of the above
15. ____ Which of the following can be used to argue that genes were acquired by horizontal transfer?
- A. clusters of genes with unusual (for the organism under examination) codon bias
 - B. some regions have G+C contents that are different than that of the genome as a whole
 - C. Many genes are more related to their archaeal homologs than to those of Bacteria
 - D. all of the above
 - E. none of the above
16. ____ A "metagenome" is ...
- A. the complete genetic complement of an environment
 - B. the complete genomes of all organisms
 - C. the complete genetic complement of an organism
 - D. the complete genomic content of a metazoan
 - E. none of the above
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Short-answer questions (5 points each)

17. Why is cloning necessary in a traditional ssu-rRNA survey of a microbial population?



18. What is proteorhodopsin?

22. Describe 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?

Essay questions (10 points each)

18. Describe *in detail* how **one** of the following microbial communities was surveyed:

- The human skin microbiome
- Chloroflexi in wastewater sludge
- Bacteria in different temperature zones of a Tibetan hot spring
- Oral microflora before and after treatment for periodontal disease

19. 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. **YOU ARE NOT ALLOWED TO USE ANY PAPER YOU'VE ALREADY DESCRIBED IN THIS EXAM!**

USE THIS PAGE TO FINISH ANY ANSWERS YOU'RE BEING LONG-WINDED ABOUT

Papers reviewed in class:

- Reysenbach AL, Wickham GS & Pace NR 1994 Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. *Appl. Env. Microbiol.* 60:2133-2199
- Huber R, et al. 1998 *Thermocrinus ruber*, gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park. *Appl. Env. Microbiol.* 64:3576-3583
- Goffredi SK, Waren A, Orphan VJ, Van Dover CL & Vrijenhoek RC 2004 Novel forms of structural integration between microbes and a hydrothermal vent gastropod from the Indian Ocean. *Appl Env Microbiol* 70:3082-3090
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI & Knight R 2009 Bacterial community variation in human body habitats across space and time. *Science* 326:1694-1697
- Björnsson L, Hugenholtz P, Tyson GW & Blackall LL. 2002 Filamentous Chloroflexi (green non-sulfur bacteria) are abundant in wastewater treatment processes with biological nutrient removal. *Microbiol.* 148:2309-2318.
- Yim LC, Hongmei J, Aitchison JC & Pointing SB 2006 Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. *FEMS Microbiol Ecol.* 57:80-91
- Sakamoto M, Huang Y, Ohnishi M, Umeda M, Ishikawa I & Benno Y. 2004 Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes. *J. Med. Microbiol.* 53:563-571.
- 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
- Beja O, et al. 2000 Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* 289:1902-1906.
- Nelson KE, . . . Venter JC, Fraser CM. 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. *Nature* 399:323-329
- Venter JC, et al., 2004 Environmental genome shotgun sequencing of the Sargasso sea. *Science* 304:66-74.
- Rasmussen, B. 2000 Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature* 405:676-679

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

- Wolfe-Simon F, et al. 2010 A Bacterium that can grow by using arsenic instead of phosphorous. *ScienceXpress* 10.1126
- Drancourt, M., Aboudharam, G., Signoli, M., Dutour, O., and Raoult, D. 1998 Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: An approach to the diagnosis of ancient septicemia. *Proc. Natl. Acad. Sci. USA* 95:12637-12640.
- Drancourt, et al., 2004 *Emerg Inf Diseases* 10:1585 Genotyping, Orientalis-like *Yersinia pestis*, and Plague Pandemics.
- Soto C and Castilla J 2004 The controversial protein-only hypothesis of prion propagation. *Nature Medicine* 10:S63-S67
- Somerville RA 2002 TSE agent strains and PrP: Reconciling structure and function. *TiBS* 27:606-612
- Murgia C, Pritchard JK, Kim SY, Fassati A, and RA Weiss 2006 Clonal origin and evolution of a transmissible cancer. *Cell* 126:477-487
- Fierer N, Hamady M, Lauber CL and R Knight 2008 The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *PNAS* 105:17994-17999

