

Final Exam

MB 451 : Microbial Diversity
Spring 2010

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

Signed : _____ Date : _____

Name : _____

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

Multiple choice (2 points each)

- _____ 2. *Thermocrinus ruber* is . . .
- A. the pink filaments of Octopus Spring
 - B. the esophageal gland symbiont of the scaly snail
 - C. the organism proteorhodopsin was discovered in
 - D. a grazing protist
 - E. the source of *Tru*-value DNA polymerase
- _____ 3. What specific evidence was presented to demonstrate that the scaly snail is nourished by autotrophic symbionts?
- A. the iron sulfide plating of the scales
 - B. the absence of an operculum
 - C. the absence of a functional digestive tract
 - D. the esophageal gland contains a single symbiont
 - E. the isotope ratios of snail tissue
- _____ 4. Which best describes a "progenote"?
- A. an organism in which genotype and phenotype aren't yet tightly linked
 - B. the last common ancestor
 - C. a genome sequence before final contig assembly
 - D. the RNAs that made up to RNA World
 - E. the metabolic activities that preceded cellular life
- _____ 5. The last of the sterilizing impacts after the formation of Earth was about 3.8 Bya. About how long after this do we see the first evidence of life?
- A. 20 minutes
 - B. 20 years
 - C. 20,000 years
 - D. 200 million years
 - E. 2 billion years
- _____ 6. Which of these microbial communities are the most different in composition?
- A. the same body part of different individuals
 - B. different parts of the body of a single person
 - C. the same body part of one person at different times
 - D. the right and left sides of a person
 - E. all contain the same, normal populations
- _____ 7. About how many sequences are generated in a single 454 sequencing run?
- A. 10 - 100
 - B. 100 - 1,000
 - C. 1,000 - 10,000
 - D. 10,000 - 100,000
 - E. 100,000 - 1,000,000

_____ 8. The failure of wastewater sludge to settle after aerobic digestion is thought to be caused by . . .

- A. FISH
- B. the mineralization of organics to CO₂ and CH₄
- C. *Peptostreptococcus*
- D. filamentous *Chloroflexi*
- E. grazing protists

_____ 9. In the outflow of the Tibetan hot spring, it was found that microbial diversity . . .

- A. decreased monotonically as temperature increased
- B. generally increased as temperature increased
- C. was highest at moderate temperatures (~65°C)
- D. fell dramatically at the highest temperatures
- E. was very high throughout

_____ 14. Optical tweezers were used to . . .

- A. pick out individual genomes for metagenomic analysis
- B. isolate the gene for proteorhodopsin
- C. pick individual cells out of enrichment cultures
- D. identify horizontally-transferred genes
- E. to create a 3D image of the population

_____ 11. What is a *metagenome*?

- A. the genome of a metazoan
- B. the genetic composition of a microbial community
- C. large fragments of a genome sequence
- D. an assembly of overlapping sequence reads
- E. a genome made of horizontally transferred genes

_____ 12. Proteorhodopsin is a . . .

- A. component of cyclic photophosphorylation
- B. light-driven ATP synthase
- C. light sensing response regulator
- D. light-driven Cl⁻ pump
- E. light-driven proton pump

_____ 13. A massive sulfide deposit is . . .

- A. the impact site of a carbonaceous chondrite
- B. a fossilized deep-sea hydrothermal vent
- C. the sludge that settles to the bottom of a "lagoon"
- D. the result of gingivitis from poor oral hygiene
- E. a dinosaur coprolite

_____ 10. At what stage of the t-RFLP process are the ssu-rRNAs sequenced?

- A. After PCR but before restriction digestion
- B. After separating the restriction fragments
- C. after re-amplification from the separated peaks
- D. after they are fluorescently-labeled
- E. this method doesn't require sequencing

_____ 15. In the stable-isotope probing (SIP) analysis of protists in wastewater sludge, when ¹³C-bicarbonate (CO₂) was feed to the sludge, protist ssu-rRNAs were labeled. How did this happen? The CO₂ was . . .

- A. taken up directly by autotrophic protists
- B. co-metabolized by Bacteria, and the resulting organics were absorbed by the protists
- C. converted to acetate by autotrophic Bacteria, and the protists absorbed the acetate
- D. taken up by autotrophic Bacteria, who were eaten by the protists
- E. Trick question! The ¹³C label never showed up in the protists in these experiments

_____ 16. What reason is there to believe that the *Burkholderia* sequence in the Sargasso Sea metagenome is a contaminant and not really from the sea?

- A. *Burkholderia* has not otherwise been seen there
- B. The *Burkholderia* sequences come from a single filter
- C. There is little or no heterogeneity in the sequence
- D. it's from a human pathogenic strain
- E. all of the above

A student, after spending the week of exams partying instead of studying, turns in his abysmal test with a \$100 bill clipped to the last page and a note saying "A dollar a point".

When he picks up the graded exam he finds he's received a score of a 27 - and clipped to the last page is \$73 change.

Short answers (5 points each)

17. Diagram how a typical molecular phylogenetic analysis of a microbial community is performed.

Microbial community



18. What is a *chimera*, and how are they generated?

19. What is "Unifrac"?

20. The authors of the *Thermotoga maritima* genome sequence paper searched for matches to each of its genes in GenBank, and in many cases found the closest match was in Archaea rather than Bacteria. They argued from this that these genes must have been horizontally transferred from Archaea to *Thermotoga*. Why is this not necessarily true?

21. What is the RNA World hypothesis?

22. DGGE and cesium-TFA centrifugation separate ssu-rRNAs or ssu-rRNA PCR products. Given that the molecules being separated are all very close to the same size, on what basis do these methods separate them?

23. How can the metabolic capabilities of an organism be determined (or at least proposed) from its genome sequence?

Essay questions (10 points each)

24. Describe *in detail* one method used to examine microbial communities. Be sure to include its strengths and weaknesses relative to other methods, and what kind of information it does and does *not* produce.



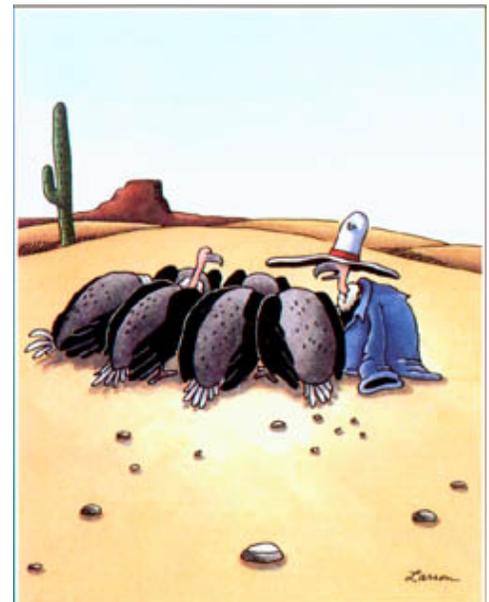
25. Describe *in detail* one of the papers we've talked about since the second midterm exam. Be sure to include the hypothesis/problem/question, approach, results, and interpretation. A list of these papers can be found on the last page of this exam. If you choose to discuss the review by Carl Woese (*Interpreting the universal phylogenetic tree*), be sure to discuss more than one of the major points made, and the evidence to support them.

26. Buruli is a necrotic disease of the skin and surrounding soft tissue and bone, caused by *Mycobacterium ulcerans*. Very common in central and western Africa, this disease is rarely fatal but causes debilitating disfigurement. *M. ulcerans* cannot be reliably grown in culture, but its ssu-rRNA sequence is known. Epidemiological evidence suggests that Buruli is transmitted to humans by the bites of aquatic arthropods, but *which* arthropod specie(s) has not been determined. Given that there are thousands of species of aquatic arthropods in the affected area, how would you go about trying to identify which specie(s), if any, transmit this disease? There is no need to describe how the methods you'd use work, but be sure to provide any particulars such as probes, labels, primers, &c, and also justify your approach by explaining why it is better than alternative approaches.

Cheat Sheet

Papers we've read since the second midterm:

1. *Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park.* Reysenbach AL, Wickham GS & Pace NR 1994 Appl. Env. Microbiol. 60:2133-2199
2. *Thermocrinus ruber, gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park.* Huber R, et al. 1998 Appl. Env. Microbiol. 64:3576-3583
3. *Novel forms of structural integration between microbes and a hydrothermal vent gastropod from the Indian Ocean.* Goffredi SK, Waren A, Orphan VJ, Van Dover CL & Vrijenhoek RC 2004 Appl Env Microbiol 70:3082-3090
4. *Bacterial community variation in human body habitats across space and time.* Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI & Knight R 2009 Science 326:1694-1697
5. *Filamentous Chloroflexi (green non-sulfur bacteria) are abundant in wastewater treatment processes with biological nutrient removal.* Björnsson L, Hugenholtz P, Tyson GW & Blackall LL. 2002 Microbiol. 148:2309-2318.
6. *Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress.* Yim LC, Hongmei J, Aitchison JC & Pointing SB 2006 FEMS Microbiol Ecol. 57:80-91
7. *Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes.* Sakamoto M, Huang Y, Ohnishi M, Umeda M, Ishikawa I & Benno Y. 2004 J. Med. Microbiol. 53:563-571.
8. *Identification of ciliate grazers of autotrophic Bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing.* Moreno Am, Matz C, Kjelleberg S & Manefield M 2010 AEM 76:2203-2211
9. *Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea.* Beja O, et al. 2000 Science 289:1902-1906.
10. *Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of Thermotoga maritima.* Nelson KE, ..., Venter JC, Fraser CM. 1999. Nature 399:323-329.
11. *Environmental genome shotgun sequencing of the Sargasso sea.* Venter JC, et al., 2004 Science 304:66-74.
12. *Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulfide deposit.* Rasmussen, B. 2000 Nature 405:676-679
13. *Interpreting the universal phylogenetic tree.* Woese CR 2000 PNAS 97:8392



Hey, look at me, everybody! I'm a cowboy! ... Howdy, howdy howdy!