MB 451 : Microbial Diversity : Midterm Exam #2

Honor Pledge: *I have neither given nor received unauthorized aid on this test.*

Signed _____Key_____ Date __3/20/06_____

Print name ___**KEY**_____

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

1. __Archaea_____
2. __Bacteria_____
3. __Eukarya_____

2) __C__ The "Big Tree of Life" based on analysis of the ssu-rRNA is... (2 points)
A. subjective and qualitative
B. correct and unambiguous
C. objective and quantitative
D. outdated and quaint
E. all of the above

3) __A__ The "Big Tree of Life" has been rooted using... (2 points)
A. analysis of ancient duplicated genes
B. auxin and hydroponics
C. the fossil record
D. the use of an outgroup
E. all of the above

4) __A__ The "Big Tree of Life" is affected by horizontal transfer of genes in this way: (2 points)
A. nobody really knows
B. no significant affect at all
C. it renders the tree largely invalid
D. by creating hybrid plants and animals
E. none of the above

5) __D__ In the symbiosis between *Chlorobium* and an uncultivated beta-proteobacterium, the beta-proteobacterium provides _____ to *Chlorobium*: (2 points)
A. NADPH and CO2
B. photosynthesis
C. sulfur granules
D. motility and phototaxis
E. all of the above

6) __E__ An ssu-rRNA with sequences from one organism at one end, but a different organism at the other end, is called a: (2 points)
A. sleipnir
B. hydra
C. selkie
D. sphinx
E. none of the above

7) __A__ The 2 major genera of Bacteria found in human feces in ssu-rRNA molecular phylogenetic surveys are: (2 points)
A. *Bacteroides* & *Clostridium*
B. *Porphyromonas* & *Streptococcus*
C. *Escherichia* & *Bacteroides*
D. *Coprothermobacter* & *Verrucomicrobium*
E. none of the above

8) __B__ Bulking and foaming of wastewater during treatment is caused by... (2 points)
A. an excess of nitrogenous and phosphate-containing organics
B. filamentous green non-sulfur Bacteria
C. biofilm-producing pseudomonads
D. the production of carbon dioxide and methane during anaerobic digestion
E. none of the above

9) __E__ *Taq* polymerase is... (2 points)
A. the RNA polymerase from *Thermus aquaticus*
B. the DNA polymerase from *Pyrococcus furiosus*
C. the polyphosphate polymerase from *Thermomicrobium roseum*
D. the taquanoic acid polymerase from *Thermocrinus ruber*
E. none of the above

10) __A__ The axial fiber of spirochaetes is comprised of... (2 points)
A. flagella
B. actin and tubulin
C. chromatin
D. magnetite beads
E. none of the above

11) __C__ The major classes of proteobacteria are... (2 points)
A. deinococci, *Thermus* & relatives
B. Crenarchaea, Euryarchaea, Korarchaea
C. alpha, beta, gamma, delta, epsilon
D. purple photosynthesizers, heterotrophs, hydrogen oxidizers
E. none of the above

12) __E__ Phenotypic groups found in the proteobacteria include... (2 points)
A. purple photosynthesizers
B. hydrogen oxidizers
C. heterotrophs
D. sulfur oxidizers
E. all of the above

13) __D__ The rhodopsins found in halophilic archaea are... (2 points)
A. sensory opsins
B. chloride pumps
C. proton pumps
D. all of the above
E. none of the above

14) __E__ The electron transport chain is driven by... (2 points)
A. oxidation of NADPH and reduction of O2
B. oxidation of sulfide and reduction of oxygen
C. oxidation of NADPH and reduction of sulfate
D. oxidation of chlorophyll and reduction of chlorophyll
E. all of the above

15) __D__ The microbial community from which *Thermocrinus ruber* was isolated was... (2 points)

A. The grey filaments of Nakabusa hot springs
B. digested wastewater sludge
C. the gingiva of dental patients teeth
D. the pink filaments of Octopus Spring
E. none of the above

16) Give an example *other than one described in the **papers** discussed in class* of the phylogenetic identification of an unculturable organism using molecular phylogenetic analysis: (2 points)

Some examples:

- The sulfur-oxiding symbionts of the giant tube worm *Riftia* and vent clam *Calyptogena*
- The causitive Corynebacterial populaations that cause non-bacterial prostatitis
- *Wolbachia*
- *Buchnera aphidicola*
- Magnetotactic Bacteria
- *Cenarchaeum symbiosum*
- *Bartonella bacilliformis*
- *Epulopiscium fishelsonii*

17) List (a) one major group of Bacteria for which there are few cultivated representatives, and (b) one major group of Bacteria for which there are *no* cultivated representatives. (2 points)

a. Some examples: *Acidobacterium* & relatives, *Verrucomicrobium* & relatives, green non-sulfur Bacteria, *Thermodesulfobacterium, Coprothermobacter, Dictyoglomus, Nitrospira* & relatives, *Synergistis, Fibrobacter, Flexistipes, Fusobacterium*

b. Some examples; OP11, OP9, OP5, WS1, OP10, OP3, OS-K, OP8, Termite group I, Marine group A, TM6, WS6, TM7

18) List three genera from each of these phylogenetic groups of Bacteria. (18 points)

Aquifex & relatives	*Aquifex, Hydrogenobacter, Hydrogenobaculum, Hydrogenivirga, Thermocrinus, Hydrogenothermus, Persephonella, Sulfruihydrogenobium, Balnearium, Desulforobacterium, Thermovibrio*
Bacteroids	*Bacteroides, Flavobacteruim, Cytophaga, Chlorobium, Chlorobaculum, Chloroherpton, Clathrochloris, Pelodictyon, Prosthecochloris, Porphyromonas, Prevotella*
Green non-sulfur Bacteria	*Chloroflexus, Chloronema, Roseiflexus, Heliothrix, Oscillochloris, Herpetosiphon, Anaerolinea, Caldilinea, Chlorothrix, Dehalococcoides, Kouleothrix, Thermomicrobium, Sphaerobacter*
Spirochaetes	*Spirochaeta, Treponema, Borellia, Leptospira*
Gamma proteobacteria	*Escherichia, Enterobacter, Citrobacter, Erwinia, Klebsiella, Proteus, Salmonella, Shigella, Yersinia, Azotobacter, Pseudomonas, Beggiotoa, Chromatium*
Beta proteobacteria	*Nitrosospira, Nitrosomonas, Rhodocyclus, Neisseria, Bordetella, Burkholderia, Oxalobacter, Hydrogenophilus, Thiobacillus, Neisseria, Gallionella, Spirillum, Azoarcus, Dechloromonas, Ferribacterium, Petrobacter, Propionivibrio, Quadricoccus, Rhodocyclus, Thauera, Zoogloea, Thauera*

19) Describe any one organism covered in this class so far. (10 points)

Example:

Genus	*Thermocrinus*
species	*ruber*
phylogenetic group	*Aquifex* & relatives
habitat	hot springs
carbon source	CO2
energy source	H2 + O2 -> H2O
approximate growth temp	80°C
morphology : (text or drawing)	
	pink, rods with polar flagella or filamentous
another trait	microaerophilic
another trait	very closely related to EM17

20) Describe *another* organism (*not* in the same major phylogenetic group as the one you used in the previous question) covered in this class so far. (10 points)

Example:

Genus	*Deinococcus*
species	*radiodurans*
phylogenetic group	*Deinococcus/Thermus* group
habitat	cloud droplets?
carbon source	organics
energy source	organics
approximate growth temp	mesophilic
morphology : (text or drawing)	
	tetrads of cocci
another trait	Division via septal "curtains"
another trait	extremely resistant to ionizing radiation

21) Describe the question/problem, approach, results and conclusion of any one of the papers discussed in class. (15 points)

Example:

Direct survey of ssu-rRNA from human feces

Purpose: To perform a census of the normal gut flora of humans.

Approach: They isolated DNA from a fecal sample (from a healthy human male) , amplified rDNA by PCR using universal bacterial-spcific primers, clone the rDNA, sequenced nearly 300 clones, and analysed them phylogenetically.

Results: About 1/3rd of the rDNA clones were from various members of the genus *Bacteroides*, nearly half were from relatives of *Clostridium coccoides*, and another 20% were from relatives of *Clostridium leptum*. The remaining 5% were a mixture of Firmicutes (*Streptococcus, Mycoplasma, Sporomusa*, and other *Clostridium*), and a single sequence related to *Verrucomicrobium*.

Conclusion: The human colon (and therefore fecal) flora is predominated by Firmicutes, and particularly members of the genus *Clostridium*, and members of the genus *Bacteroides*. The organisms we usually think of as normal gut flora, *e.g. E. coli* & other "enterics", lactobacilli, etc, must make up such a trival fraction of the gut flora that they were not detected.

22) Briefly describe one thing, from any of the required papers *other* than the one you answered with in the previous question, that was not discussed in class. (5 points)

Example:

In the Nakagawa paper surveys the microbial composition of a japanese hot spring, the authors also described the fact that the grey streamer community that grows at ca. 65°C, which seem to be rich in members of the genus *Thermodesulfobacterium* on the basis of the ssu-rRNA analysis, are capable of the reduction of sulfide to sulfate.

23) Briefly describe ***one*** of the following techniques: DGGE, t-RFLP, SIP. (10 points)

Example: DGGE

DGGE starts out like almost molecular phylogenetic analysis does; by the isolation of DNA from environmental samples, followed by PCR of ssu-rRNA genes. Rather than cloning and sequencing from this pool of genes, however, they are first separated into unique sequences based on their denaturation properties.

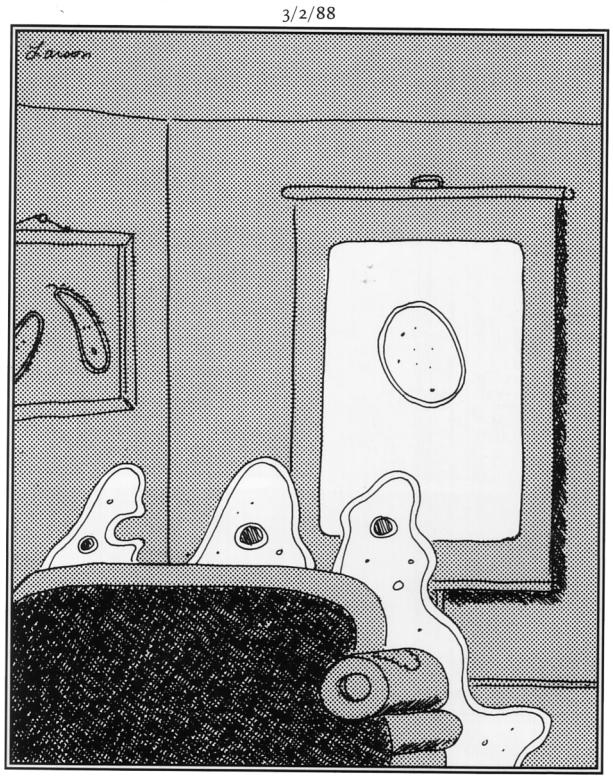
DGGE is carried out in polyacrylamide gels in which the concentration of urea and formamide increases from top to bottom in the gel; i.e. the gel contains a gradient of denaturants. (Remember that denaturation of DNA means separation of the two strands.) The PCR-amplified ssu-rDNA is loaded in wells at the top of the gel, where the concentration of urea/formamide is too low to denature the DNA. As the ssu-rDNA migrates down the gel during electrophoresis, the concentration of urea/formamide increases until, at some point, it is high enough to denature the DNA. At this point, the ssu-rDNA band essentially stops moving (it slows way down). Because every ssu-rDNA sequence will have a different denaturation point, they will denature at different levels of the gel and separate into distinct bands despite the fact that the ssu-rDNAs in all of the bands are all the same size.

24) Suppose you've done a molecular phylogenetic "survey" of some environment using the standard ssu-rRNA PCR/clone/sequence process, but you've surprised by the sequences you get. You have reason to think that perhaps the sequences are *not* representative of the original microbial population. How might you go about testing this? (5 points)

Example:

By making fluorescent probes to each specific to each sequence and probing the original sample by FISH. By counting total cells and those stained with each probe, you should be able to assess the frequency of each organism found in the phylogenetic survey, and then compare these to the numbers seen in the original survey.

(You could also use t-RFLP or DGGE, but these will also have the same potential biases of PCR amplification.)



"No, wait! *That's* not Uncle Floyd! Who is that? ... Crimony, I think it's just an air bubble!"