Midterm Exam #2

MB 451
Microbial Diversity

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

Signed : __*Key*_____ Date : __*3/19/07*_____

Name : __*Key*_____

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

Archaea, Bacteria & Eukarya

Multiple choice (2 points each, 22 points total)

2. _*B*__ The Universal Tree of Life was rooted by phylogenetic analysis of ...
   - *A. viral polymerase genes*
   - *B. ancient duplicated genes*
   - *C. antibiotic resistance genes*
   - *D. ribosomal RNA genes*
   - *E. organellar genes*

3. _*C*__ "Horizontal" or "lateral" gene transfer is the acquisition of a gene from ...
   - *A. your siblings*
   - *B. your direct ancestors*
   - *C. somewhere other than your ancestors*
   - *D. elsewhere in the genome*
   - *E. none of the above*

4. _*A*__ A "phylogenetic probe" is ...
   - *A. a fluorescently-labeled oligonucleotide*
   - *B. a type of molecular phylogenetic analysis*
   - *C. a type of microelectrode*
   - *D. a phylotype*
   - *E. none of the above*

5. _*E*__ Molecular phylogenetic analysis can be used to ...
   - *A. identify predominant organisms in a population*
   - *B. assess the relative abundance of organisms*
   - *C. Identify or count specific organisms*
   - *D. Identify unculturable organisms*
   - *E. all of the above*

6. _*E*__ When analyzing rRNA sequences from community DNA, the PCR-amplified DNA must be cloned before sequencing because ...
   - *A. the DNA is too hazardous to handle uncloned*
   - *B. the RNA sequences have to be converted to DNA*
   - *C. all of the molecules are the same size*
   - *D. otherwise the probe won't hybridize*
   - *E. the mixture of sequences must be separated out*

7. _*A*__ An ssu-rRNA sequence from 2 different organisms fused together is called a ...
   - *A. Chimera*
   - *B. rat-FISH*
   - *C. Cerberus*
   - *D. Banshee*
   - *E. Sphinx*

8. _E__ The electron transport chain ...

A. separates a redox reduction into half-reactions

B. generates a proton gradient

C. generates an electrical gradient

D. contains both electron and hydrogen carriers

E. all of the above

9. _D__ Which of the following is *not* a way to fix carbon?

A. the hydroxypropionate pathway

B. the reverse TCA cycle

C. the Calvin cycle

D. the Knallgass reaction

E. all of the above can be used to fix carbon

10. _A__ "Bulking" during wastewater treatment is caused by ...

A. failure of the sludge flocs to settle

B. dilution of wastewater from hard rainfall

C. failure of methanogenesis in the "lagoon"

D. too much raw sewage entering the facility

E. none of the above

11. _E__ About how many bacterial species exist?

A. hundreds

B. thousands

C. millions

D. tens of millions

E. who knows?

12. _E__ Which of the following is *not* a way to survey (take a census of) a microbial population?

A. denaturing gradient gel electrophoresis

B. terminal restriction fragment length polymorphism

C. sequencing lots of clones from PCR-amplified rRNA from DNA extracted from environmental samples

D. flourescent in situ hybridization

E. all of the above can be used to survey populations

Fill in the blank (1 point per blank, 18 points total). There is a list of genera from the notes on page 4 for your reference.

13. List 2 genera from each of these phylogenetic groups of Bacteria.

Aquifex & relatives	*e.g. Aquifex (Duh!)*	*e.g. Hydrogenobacter*
Deinococcus & relatives	*e.g. Deinococcus (Duh!)*	*e.g. Thermus*
Bacteroids	*e.g. Bacteroides*	*e.g. Flavobacterium*
Green non-sulfur Bacteria	*e.g. Chloroflexus*	*e.g. Herpetosiphon*
Spirochaetes	*e.g. Treponema*	*e.g. Borrelia*
Gamma proteobacteria	*e.g. Escherichia*	*e.g. Chromatium*
Beta proteobacteria	*e.g. Thaeura*	*e.g. Bordetella*

14. *e.g. Verrucomicrobium & relatives* and *e.g. Acidobacterium & relatives* are major phylogenetic groups of Bacteria with *few* cultivated representatives.

15. _*e.g. OP11*_ and _*e.g. OS-K*_ are major phylogenetic group of Bacteria with *no* cultivated representatives.

Short answer (5 points each)

Choose 5 out of the 7 phylogenetic groups listed below, and briefly describe one organism in that group. IF YOU ANSWER MORE THAT 5 OF THESE 7 QUESTIONS, ONLY THE FIRST 5 WILL BE GRADED. There is a list of genera from the lecture notes on the bottom of the next page for your reference.

16. *Aquifex* & relatives group

Name (genus) : *Thermocrinus*

Morphology : *filamentous or rods*

Energy & carbon sources : *carbon from CO2 and energy from hydrogen oxidation*

Habitat : *hot springs*

Something else about it : *These are the famous "pink filaments" from Octopus Spring!*

17. Bacteroids

Name (genus) : *Bacteroides*

Morphology : gliding rods

Energy & carbon sources : *sugar (saccharolytic)*

Habitat : *the human gut*

Something else about it : *These are one of the major components of the gut flora*

18. Green non-sulfur Bacteria

Name (genus) : *Chloroflexus*

Morphology : *flexible filaments*

Energy & carbon sources : *carbon from CO2, energy from light*

Habitat : *hot spring mats*

Something else about it : *carbon fixation uses the hydroxypropionate pathway*

19. *Deinococcus* & relatives

Name (genus) : *e.g. Thermus*

Morphology : rods

Energy & carbon sources : *organics (it's a heterotroph)*

Habitat : *hot springss - pink filaments mats*

Something else about it : *It's the source of Taq polymerase used in PCR*

20. Spirochaetes

Name (genus) : *Borrelia*

Morphology : *"spirochaete" - helical, wrapped around the axial fiber*

Energy & carbon sources : *sugar (saccharolytic)*

Habitat : *it's a parasite - insects & mammals*

Something else about it : *The causative agent of Lyme's Disease*

21. Gamma-proteobacteria

Name (genus) : *e.g. Beggiotoa*

Morphology : *filamentous*

Energy & carbon sources : *energy from sulfide oxidation, carbon from CO2*

Habitat : *aquatic - sulfur springs*

Something else about it : *motile by gliding*

22. Beta-proteobacteria

Name (genus) : *e.g. Neisseria*

Morphology : *cocci*

Energy & carbon sources : *organics (heterotrophic)*

Habitat : *animals bodies (these are parasites)*

Something else about it : *include the causative agents of gonnorhoeae and meningitis*

LIST OF GENERA EXTRACTED FROM THE LECTURE NOTES

Acidobacterium
Anaerolinea
Aquifex
Axinella
Azoarcus
Azotobacter
Bacteroides
Balnearium
Bartonella
Beggiotoa
Bordetella
Borrelia
Buchnera
Burkholderia
Caldilinea
Calyptogena
Cenarchaeum
Chlamydia
Chlorobaculum
Chlorobium
Chloroflexus
Chloronema
Chlorothrix
Chloroherpeton
Chromatium
Citrobacter
Clathrochloris
Clostridium
Coprothermobacter
Corynebacterium
Cytophaga
Dechloromonas
Dehalococcoides
Deinococcus
Desulfurobacterium
Dictyoglomus
Enterobacter
Epulopiscium
Erwinia
Escherichia
Ferribacterium
Fibrobacter
Flavobacterium
Flexistipes
Fusobacterium
Gallionella
Geothrix
Heliothrix
Herpetosiphon
Holophaga
Hydrogenobacter
Hydrogenobaculum
Hydrogenophilus
Hydrogenothermus
Klebsiella
Kouleothrix
Leptospira
Marinithermus
Meiothermus
Mycoplasma
Neisseria
Neurospora
Nitrosomonas
Nitrosospira
Oceanothermus
Opitutus
Oscillochloris
Oxalobacter
Pelodictyon
Peptostreptococcus
Persephonella
Petrobacter
Porphyromonas
Prevotella
Propionivibrio
Prosthecobacter
Prosthecochloris
Proteus
Pseudomonas
Quadricoccus
Rhodocyclus
Rickettsia
Riftia
Rochalimaea
Roseiflexus
Salmonella
Shigella
Sphaerobacter
Spirillum
Spirochaeta
Streptococcus
Sulfurihydrogenobium
Synergistis
Thauera
Thermocrinus
Thermodesulfobacterium
Thermomicrobium
Thermovibrio
Thermus
Thiobacillus
Treponema
Ultramicrobium
Verrucomicrobium
Vulcanithermus
Wolbachia
Yersinia
Zoogloea

Essay (10 points each)

CHOOSE 3 OUT OF THESE 4 QUESTIONS - IF YOU ANSWER THEM ALL, ONLY THE FIRST 3 WILL BE GRADED

23. Summarize the question/problem, approach, results and conclusion of any one of the papers discussed in class.

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-specific primers, clone the rDNA, sequenced nearly 300 clones, and analyzed 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 trivial fraction of the gut flora that they were not detected.

24. Summarize the question/problem, approach, results and conclusion of *another* paper discussed in class.

e.g. Evidence for a new type of phototrophy in the sea.

Purpose: To identify the ecological role played by the uncultivatable group SAR86.

Approach: Cloned large chunks of DNA isolated from seawater, identified a big clone (130Kbp) with a SAR86 rRNA, and sequenced the entire piece of DNA in attempt to identify genes that might give a clue about it's lifestyle.

Results: The DNA encoded a bacteriorhodopsin-like gene, which when expressed in E.coli (and supplemented with retinal) functioned in various ways as a light-driven proton pump.

Conclusion: SAR86, a very abundant gamma proteobacterium in seawater worldwide, seems to be living phototrophically, using an archaeal-like "proteorhodopsin" rather than traditional bacterial photosystems.

25. From any of the papers discussed in class, describe one aspect of the paper that was ***not*** discussed in class.

e.g. *Changes in oral microbial profiles after peridontal treatment as determined by molecular analysis of 16S rRNA genes.*

In this paper, the authors used primarily t-RFLP to characterize various oral samples before and after treatment. One aspect not discussed in class is their use of realtime PCR to demonstrate the presence of various common oral spirochaetes. What was interesting about this, and perhaps the reason they did it, was that they didn't see these to-be-expected organisms in their t-RFLP data, nor in their rRNA clone sequencing! They could be detected by realtime PCR, perhaps because these organisms, although important pathogenically, don't make up a large enough fraction of the population to be detected by t-RFLP, or by rRNA clone sequencing (given the small number of clones sequenced).

26. Describe the *purpose* and *process* (how it works) for ***one*** of the following techniques: denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (t-RFLP), or stable-isotope probing (SIP).

Example: DGGE

The purpose of DGGE is to separate rRNA PCR product mixtures into distinct bands, even though all of the different sequences are all basically the same size.

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.