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Homework 4
Due May 1st at the beginning of lecture

Instructions:
You are welcome to discuss concepts with your classmates but must compose your own answers. If you are unsure of the honor code for this course, please ask or look at the course website. http://www.its.caltech.edu/~bi1/Bi1__Micro-_to_Macro-Biology/Policies.html
The goal of this assignment is to help you understand a dense research paper. Many of the questions do not have a single correct answer. You will be given full credit as long as your answer is reasonable.
The answers must be legible and should not extend past the allotted space. Keep in mind that a few well-written sentences can give a higher score than a whole page of text.
Remember to write your full name and section number on each page.

Termites are an extremely successful group of wood-degrading organisms, known for destroying houses and crops. These small insects harness great power of cellulose destruction; even more fascinating is that the termite is not fully responsible for all of this damage. All known termite species form obligate, nutritional mutualisms with diverse gut microbial species found nowhere else in nature. These bacteria are able to degrade complex polysaccharides used in plant cell walls into simple sugars, which are then used for energy. Within each colony of termites, there are three levels of hierarchy. The reproductives are responsible for carrying on the termite lineage. Primary reproducers include the Queen, the King, and swarmer termites that are sent out to start new colonies. Secondary reproductives support the Queen and are the primary source of egg production in established colonies. Worker termites are responsible for building and repairing the colony’s mud tubes and tunnel walls, feeding the other termites, caring for eggs, and removal of dead termites. Soldier termites defend the colony from invaders, most commonly ants.

Read the paper carefully and answer the following questions.
Read Warnecke et. Al (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*. 450:560-U17 carefully and answer the following questions.

1) In the experiments conducted in this paper, the P3 lumen bacteria were isolated from only termites of the worker group. (5 points)
    a. Why were the workers chosen to be studied over the other castes of termites in the colony? (2 sentence maximum)

The worker termites are responsible for bringing the food and feeding the rest of the termites and thus are the most involved in the digestion of cellulose. The paper is looking at the microbiota of the termite gut to identify any potential concepts for the use in biofuels, so the most relevant gut would be that of the worker.

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b. Would you expect significant differences in the gut microbiota of termites from different groups? (2 sentence maximum)

Yes – the different groups have specific roles they fill, and different diets between the castes as the workers feed the rest of the castes, they would likely have different microbiota.

2) In your own words and in 4 or fewer sentences, state the goal and significance of the research in the paper. (4 points)

The paper aims to generate a large, and diverse list of bacterial genes that are involved in the processes of breaking down cellulose. The metagenomic data provided in the paper gives clues as to how the termite uses its microbiota to digest wood – this knowledge can be used to create novel schemas for the production of biofuels from cellulose.

3) Cheap and easy energy from renewable earth-abundant resources is the driving force of the modern energy revolution. Although the concept of utilizing cellulose, the most abundant organic material on our planet, would be revolutionary, could you think of any downsides to harnessing such enzymatic power? (4 points) (4 sentence maximum)
   - Would need larger areas to support the agriculture needed to create the plants for the biofuels.
   - Deforestation is already a problem, so using tree matter for fuel would increase deforestation.
   - Other ideas that are plausible accepted.

4) Why was the 16S rRNA used for the sequencing of the microbiota community of the P3 lumen of the termite? (4 points) (3 sentence maximum)

The 16s rRNA serves a vital function and thus all species maintain a copy of the gene. The mutation rate of the 16s rRNA is slow due to the necessity of it's conservation so the mutations that do occur can be used to identify a variety of species within a microenvironment.

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5) The Leadbetter lab used Sanger sequencing, PCR, and bioinformatics to perform metagenomic analysis on the microbiota. (25 points)

a. The Leadbetter lab used Pfam, a database of protein families represented by multiple sequence alignments and hidden Markov models to search for glycoside hydrolases and carbohydrate binding modules. The supplementary materials provide sequences of GHs that did not have a known family in the Pfam database at the time. Given the following protein sequence, search in Pfam (http://pfam.sanger.ac.uk/ use sequence search) to see if there are any Pfam entries and list them in the space provided. [4 points]

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FYYGWYGNPQQDGQWQHWNHRVLPYGDIPLEGRLDFPGADDIGANFYPSLGSYS
SHDPEIIEQHLEMMRQAGIGVVSVSWLGADDFAARSIDFFMDKAAEKGLQINFHIEP
NYRSAEEFHAIIAELMRKFGTHPALYRYRGKPLYYVYDSYKMPVSEWQKLLLPGGE
LSLRTPELDGQFIGLWVNQGEEAFFLDTGFDGFYTYFASEGFVWGSTSTNWPYLA
GWASRHGKLFIPSVGPGYADDRIRPWNGANFKAREQGRYYDRMFSQALNTKPDIV
TITSFNEWHEGTQIEPAVVKQLPDYRYLDYGDLPEDYYLQRTLD WSRKLSAIPVNS
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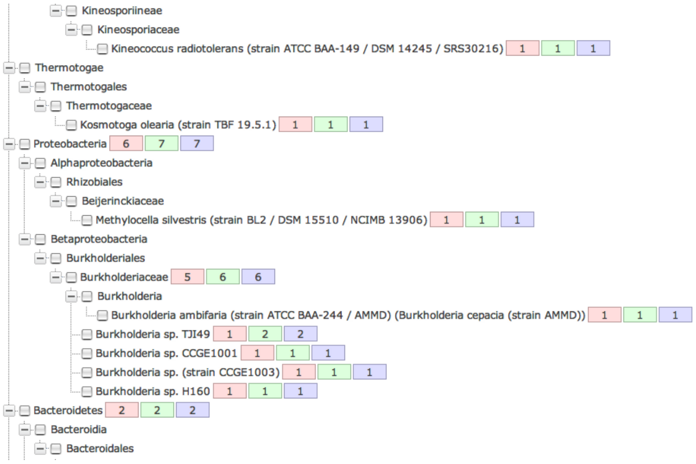
Family	**Description**
Glyco_hydro_71	Glycosyl hydrolase family 71
Glyco_tran_WbsX	Glycosyltransferase WbsX
Glyco_tran_WbsX	Glycosyltransferase WbsX

b. In Pfam, click on the family name and select **SPECIES** on the left side of the page. Using the sunburst or tree (HINT: tree format will be easier to read) to view distribution of the protein family across species and figure 1b from the paper, which phylum of bacteria is it most likely that the protein sequence came from in the termite's hind gut? Paste the section of the tree/sunburst you used to determine this. [6 points]

Proteobacteria



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        ☐ Kineosporiineae
          ☐ Kineosporiaceae
            ☐ Kineococcus radiotolerans (strain ATCC BAA-149 / DSM 14245 / SRS30216) 1 1 1
☐ Thermotogae
  ☐ Thermotogales
    ☐ Thermotogaceae
      ☐ Kosmotoga olearia (strain TBF 19.5.1) 1 1 1
☐ Proteobacteria 6 7 7
  ☐ Alphaproteobacteria
    ☐ Rhizobiales
      ☐ Beijerinckiaceae
        ☐ Methylocella silvestris (strain BL2 / DSM 15510 / NCIMB 13906) 1 1 1
  ☐ Betaproteobacteria
    ☐ Burkholderiales
      ☐ Burkholderiaceae 5 6 6
        ☐ Burkholderia
          ☐ Burkholderia ambifaria (strain ATCC BAA-244 / AMMD) (Burkholderia cepacia (strain AMMD)) 1 1 1
        ☐ Burkholderia sp. TJI49 1 2 2
        ☐ Burkholderia sp. CCGE1001 1 1 1
        ☐ Burkholderia sp. (strain CCGE1003) 1 1 1
        ☐ Burkholderia sp. H160 1 1 1
☐ Bacteroidetes 2 2 2
  ☐ Bacteroidia
    ☐ Bacteroidales
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c. What is a function of a large portion of this phylum's free-living members that might benefit the termites? (1 sentence maximum) [6 points]

Nitrogen Fixation

d. Based on information in the paper, why would members of the microbiota of a termite's hind-gut possessing this functionality be important? i.e. How does the bacteria increase the fitness of the termite by filling this niche?(3 sentence maximum) [9 points]

Wood is a poor source of nitrogen, so a symbiosis with a bacterium that can fix the nitrogen would benefit the termite by allowing the termite to gain and utilize nitrogen from its wood-based diet.

6) The bacteria in the hind-gut of termites are motile, a very uncommon occurrence compared to humans' non-motile gut inhabitants. (7 points)

a. List two ways in which motility may be advantageous for these bacteria.

1. The motile bacteria can move around within the gut of the termite.
2. Movement could allow the bacterium to more effectively seek and obtain their food source within the hind-gut.
3. Other plausible ideas accepted.

b. Can you think of any disadvantages? (5 sentence maximum)

This is a conceptual question. Plausible ideas accepted.
Ideas:

The need to use energy to generate the motility could cause strain on the bacterium if there is not enough food source within the hind-gut.

With motility there could be the loss of the ability to occupy a specific niche within the hind-gut of the termite.