Strain Carbon Sourcing and Antibiotic Resistance Protocol
02/27/2020

Objective: In this experiment we will extend our genetic characterization of strains to their phenotypic properties. Specifically, we will measure the ability of each strain to grow on a variety of carbon sources as well as their ability to resist antibiotics.

Approach: We will grow each of six strains of bacteria in the presence of 3 antibiotics and 4 different carbon sources for growth. We will then use light scattering ("optical density") measurements to determine whether or not the cells grew in the different conditions.

Growth conditions:

(1) You will be supplied with media which contain the following carbon sources:
   (a) M9 minimal medium + arginine (0.5% w/v) = C1
   (b) M9 minimal medium + galactose (1% w/v) = C2
   (c) M9 minimal medium + phenylalanine (1% w/v) = C3

In each of these "minimal media" cells can ONLY grow if they are able to consume the carbon source supplied since it is the only source of carbon available.

(2) You will also be supplied with "rich media" (= LB, plenty of nutrients) supplemented with different antibiotics:
   (a) LB + chloramphenicol (35 ug/ml) =A1
   (b) LB + ampicillin (50 ug/ml) = A2
   (c) LB + kanamycin (50 ug/ml) =A3

In these conditions cells can only grow if they are able to resist the antibiotics in that conditions.

Note: these antibiotics may be toxic. **Wear** lab coats and gloves when handling.

Step 1: **Setup cultures**. You will be provided with 6 media and overnight cultures of all 5 strains. In a sterile 96 well plate, construct duplicate cultures of each strain in each media condition. For example, one plate layout could be:

Conditions	C1	C2	C3	A1	A2	A3
Strain 1 replicate 1						
Strain 1 replicate 2						
Strain 2 replicate 1...						

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In each well add *199 ul* of medium + *1 ul* of overnight cell culture. Include two *200 ul* blank (medium that has not been inoculated with cells) **for each medium**. Overall, there should be 84 filled wells.

Carefully parafilm the 96 well plate and shake for 2 days at 950 rpm and 30C.

Step 2: Ask your TA to help with getting the light scattering ("optical density") measurements of the plate(s). This will output an excel file with the absorbance readout for each well. To test whether a strain grew in a particular condition, compute the optical density in that well by subtracting the optical density of the appropriate control well (blank = no cells). This should result in a string of true/false statements (growth/no growth). Compute the Hamming Distance between each strain to determine which strains are most similar. How does this compare with the phylogenetic tree that you have previously calculated?