Im9 Mutants II

Purpose:
To explore protein structure and function by making novel mutations in a protein with known structure.

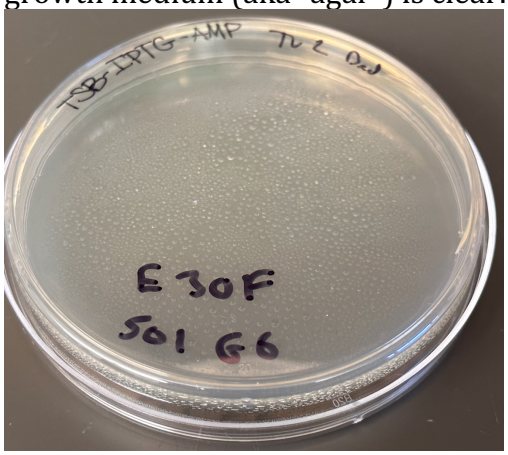
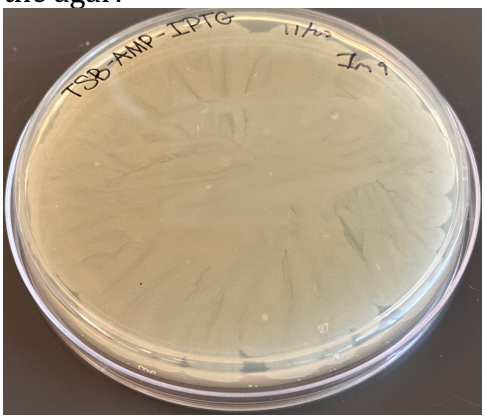
Procedure
In a previous week, you selected two mutants of the Im9 immunity protein: one mutant that you thought would have no effect on the protein ("harmless") and one that you thought would have an effect on the protein ("harmful").

Today, you will set up a test of the mutants and some control strains, to see which proteins confer resistance to Colicin E9 toxin. The results will be available next week in lab.

The four bacterial strains we'll use today are:

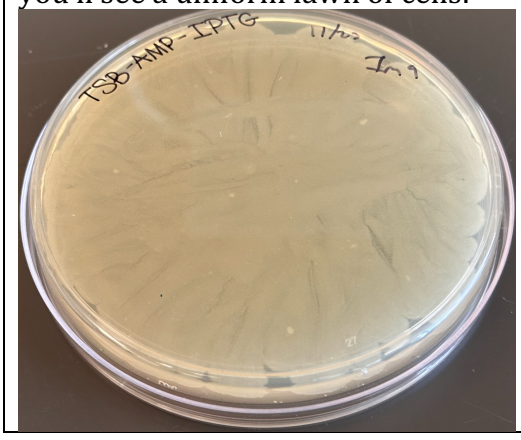
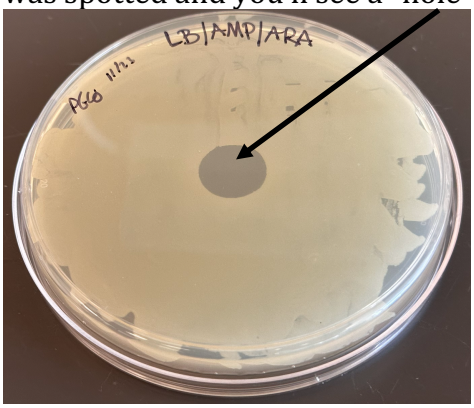
- Two *control strains*:
  - *Bacteria carrying the plasmid **pGLO***. Since this plasmid does not encode any immunity protein, it should be **killed** by the toxin.
    - This is to be sure that the toxin is still active.
  - *Bacteria carrying a plasmid with the **wild-type Im9** Gene*. This produces fully-functional Im9 protein, so it should be **immune** to the toxin.
    - This is to be sure that the toxin doesn't kill resistant cells.
- Two *experimental strains*:
  - *Bacteria carrying a plasmid with the Im9 gene with a "harmless mutation"*: We're not sure what will happen here, but if we're right, the mutant Im9 protein should be fully functional so it should be **immune** to the toxin.
  - *Bacteria carrying a plasmid with the Im9 gene with a "harmful mutation"*: We're not sure what will happen here, but if we're right, the mutant Im9 protein should be non-functional, so it should be **killed** by the toxin.

We'll test the different strains by making a "lawn" (a uniform layer of live bacteria) on petri dishes. We do this by spreading a suspension of bacteria on the plate and letting it grow overnight. This is shown below:

Before you put the bacteria on the plate, the growth medium (aka "agar") is clear: 	Once the bacteria have grown into a "lawn", you can see the lawn as a film of bacteria on the agar: 

Today, you will spread the bacteria on the plate. At this point, there will be too few bacteria to be visible. Your TA will spot a drop of toxin in the middle of the plate. We will let them grow overnight at 37C. The bacteria will grow unless they're killed by the toxin.

When you get the results back in a week or so, they'll look like this:

If the Im9 protein is present and functional, the cells will be **<u>immune</u>** to the toxin and you'll see a uniform lawn of cells: 	If the Im9 protein is absent or non-functional, the cells will be **<u>killed</u>** by the toxin where it was spotted and you'll see a "hole" in the lawn: 

Procedure

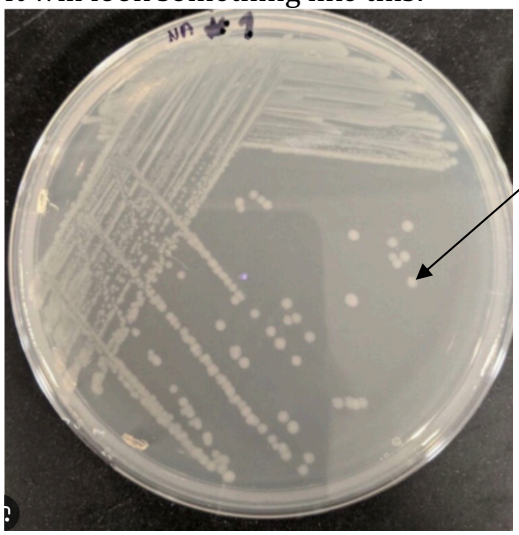
Since all bacteria look alike, you'll have to be VERY CAREFUL to label our plates and check your work carefully – otherwise we will not be able to interpret our results.

To help with this, each group will only spread out one strain and each column of lab tables will do the same strain.

1) Your TA will give each group a plate with appropriate growth medium for your group's strain:
- For bacteria carrying pGLO, the plate should be LB-amp-Ara
- For bacteria carrying a plasmid with wild-type or mutant Im9, the plate should be TSB-amp-IPTG

Label the bottom (not the lid) of your plate with:
- Your group name
- The date
- The plasmid your strain is carrying

2) Your TA will pass to your column of lab tables a plate with colonies of your strain growing on it. It will look something like this:



Each of these spots is a pile of roughly 1 billion bacteria called a "**colony**".

3) You will have a microfuge tube with 0.5ml of LB growth medium in it. Have it out – **but keep it closed** – and ready for the next step.

4) Using a sterile loop, very carefully scoop up 2-3 colonies, open the tube, and swish the loop around in the LB until most of the bacteria are no-longer on the loop. Discard the loop and cover the tube.

5) Vortex the tube until you have a uniform suspension of your bacteria.

6) Pour as much of the suspension as you can – no need to get it all – onto the medium on the plate and spread it around with another sterile loop. Discard the loop, the tube, and cover the plate.

7) Keep your plate with its cover side down on your bench until the liquid dries into the plate.

8) When your plate is dry, your TA will spot on a drop of toxin in the middle of the plate.

9) Give your plate to your TA and we'll incubate it at 37C overnight. You'll see the results next week.