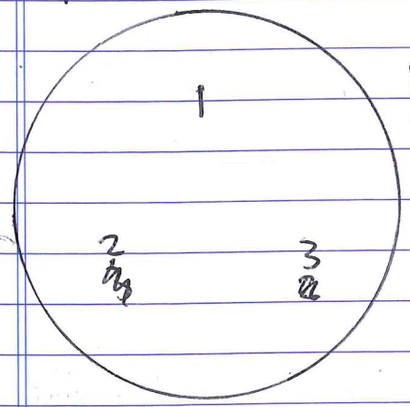
5/25/18 To figure out what is happening with my tests I am doing protein precipitation from my evolved 001 for pH 4.6 as it is a well known toxin produced. I cultured 6 ml overnight and will run 5 precipitations:

1. Angelus Super Saturated Ammonium sulfate
   - I knows it worked before.
2. My Ammonium sulfate
   - It should be the exact same as Angelus
3. 80% Solid Ammonium sulfate
4. 75% Solid Ammonium sulfate
5. 70% Solid Ammonium sulfate

These will not be rotated

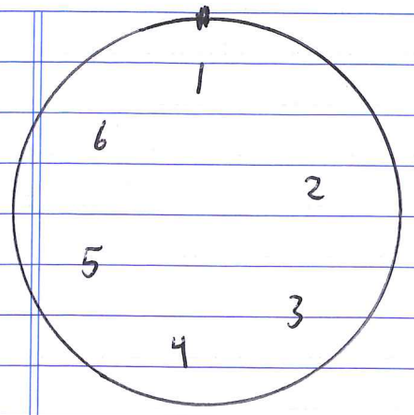
Additionally I am testing three different samples of BJHool to find and use the one that kills on the lawns it should unlike those used in the past several tests.



1- Ancestral 001
2- Smooth white
3- Yellow lumpy

5/29/18 Results:

The "ancestral" seems to produce no kill zone. At some point in my streaking to singles I must have used a contaminating strain. Need ancestral will be from Emily's 001 stock.



Precip Plate

1. Angelas Amm Sulf
2. My Amm Sulf
3. 80% Solid Amm Sulf
4. Yeast
5. 75% Amm Sulf
6. 70% Amm Sulf

5/25/18 Results:

Angelas supersaturated seems to be the best followed closely by my supersaturated and the 80% solid addition. 75% and 70% were notably smaller.

Pepsin data

Date

Data: It would appear that the pepsin had no effect whatsoever on the toxins killing ability. Maybe switch to papain or experiment more.

Pepsin Digestion

Date

6/12/18

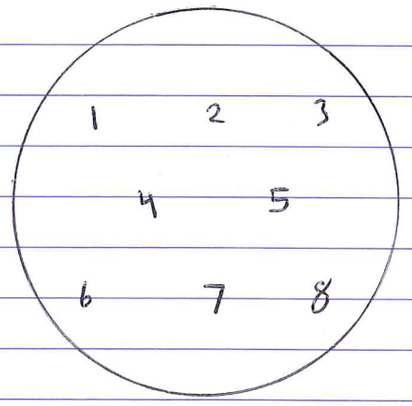
I prepared pepsin in 10mM HCl this time. I think the dd water used for the first pepsin dissolution may have been basic causing the pepsin to degrade.

~~Once the pepsin was prepared I~~

Pepsin was prepared by adding .2g of pepsin powder to a 50mL conical then filling the tube with 50 mL 10mM HCl. After being mixed and left for an hour 400 µL were taken and transferred into 10 mL of cold 10mM HCl. Both pepsin solutions will be used. They are labeled .4% and D for dilute on the toxin containing tubes.

Pepsin is added and the toxin + pepsin mix is left on the bench for the following increments of time: (.001 toxin on BT4741)

1. pepsin 4 hours
2. pepsin 3 hours
3. pepsin 2 hours
4. pepsin 1.5 hours
5. pepsin 1 hour
6. pepsin 30 min
7. 10mM HCl ~~15~~ hours
8. Nothing 0 hours - positive control



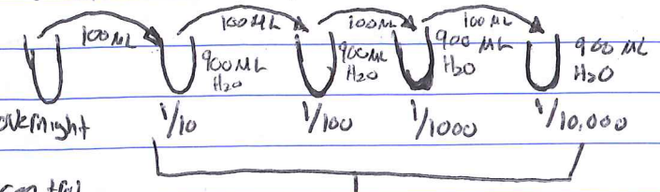
Data!

Nothing worked, yet again.

Date

Toxin Lawn

6/12 Using 4.1ml toxin precips from 001 I suspended the precipitated toxin in 600 mL pH 4.6 YPD and spread it on a pH 4.6 plate. I then allowed it to dry before plating a serial dilution of BY4741.



A control Plate with no toxin was used

Plated in triplicate.

Observations:

6/13 **18 hours**

There is a significant difference between the two plates. The toxin lawn seems to be inhibiting growth very well. The 1/100 dilution expresses this the best.

24 hours

The toxin plate has seen no significant growth while the control plate has experienced significant growth.

6/14 **40 hours**

The control plate has grown up very well while the toxin lawn still shows no good growth. Very promising

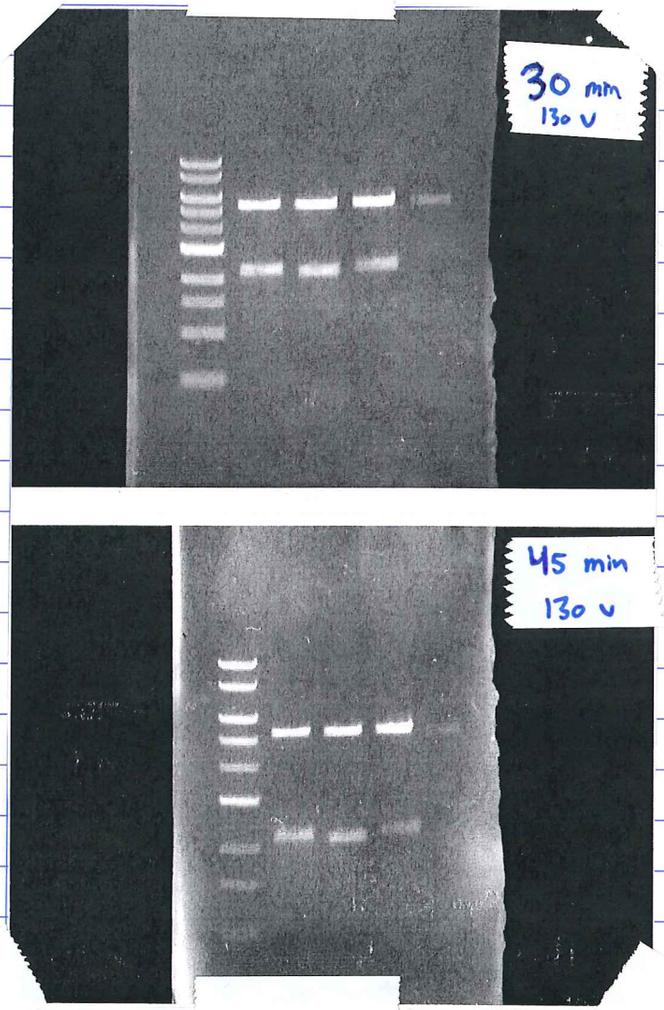
dsRNA Extraction from Evo Strains.

6/14 25 mL cultures were grown at pH 4.6 + 25°C to allow for protein precipitation as well as extractions.

Okada dsRNA Extraction used

4 mL of each sample will be placed in the well along with one sample for Angela.

My samples are from the ancestral, 4.6 evo, and pH 7 evo.



Lane 1 Ladder
Lane 2 Ancestral 001
Lane 3 pH 4.6 evolved
Lane 4 pH 7 evolved
Lane 5 Angelas

Need: 2% gel