

## Table of Contents

July 2023.....	2
Figure 1. Detection of potential laurenobiolide-resistant mutant. ....	2
Figure 2. Vials containing laurenobiolide.....	3
Future To-Do .....	6

## July 2023

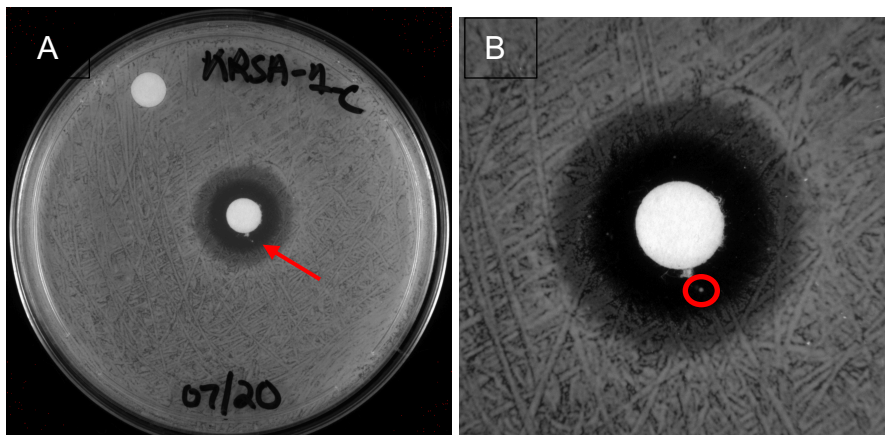
**Thursday, July 27, 2023**

**To Do:**

1. ~~Other stuff~~

**Results and Data:**

Looked at disc diffusion assay plates from Kira and Adja's experiment on 7/20. Kira had already found some potentially laurenobiolide-resistant colonies. I found a new one on the plate with just the wild-type *S. aureus*, KRSA1.



**Figure 1. Detection of potential laurenobiolide-resistant mutant.**

**A.** Plate from Adja and Kira's disc diffusion assay from 7/20/23, with lawn of KRSA1 (SA113) and disc with laurenobiolide (8 mg/mL). Arrow points to colony in zone of inhibition. **B.** Close-up view of colony (circled).

Picked out indicated colony and struck to single colony on LB, incubated O/N at 37°C.

**Friday, July 28, 2023**

**To Do:**

1. ~~Check on *S. aureus* plate, discuss with Kira~~

**Results and Data:**

Kira and I discussed a plan:

Kira will start 6 O/N cultures from the single colonies of potentially laurenobiolide(LB)-resistant cells.

I will freeze them down on Saturday

Since there is ~100 uL of LB left at 8mg/mL, I could screen a few of colonies for resistance to LB.

Asked Kira to also streak out KRSA1 (wild-type) and KRSA4 (LB-resistant) for me for controls.

**Saturday, July 29, 2023**

**To Do:**

1. Freeze down potentially laurenobiolide resistant strains
2. Pellet some of each stock, in case they are LB-resistant and we want to isolate gDNA
3. Pour plates for disc diffusion assay tomorrow
4. Patch out cells for disc diffusion assay tomorrow

**Results and Data:**

Froze down potential LB-resistant cells that Kira started O/N. Make new box (S. aureus Potential laurenobiolide<sup>R</sup>?) and store in section 8C of the -80°C freezer.

KRSA1-PLBRA

KRSA1-PLBRB

KRSA1-PLBRC

KRSA1-PLBRD

KRSA1-PLBRE

KRSA1-PLBRF

Also pellet and freeze 1 mL of cells for gDNA isolation later, if necessary.

Pulled out LB plates from 4°C to patch out cells for tomorrow. While doing so, check that we have enough LB.

!!! Not even 40 uL of laurenobiolide left!

Either scrap experiment or resuspend more laurenobiolide. Check how much we have in the freezer. There are three vials, two of which have legible labels.



**Figure 2. Vials containing laurenobiolide.**

Difficult to see labels of red vials in this photo, but one is 1.2 mg and the other is 2.3 mg. Unclear what the number of the blue-capped vial represents- if it is in ng, then it should be 12.7083 mg? I don't think we've ever received that much!

Resuspend and combine the drug from the two red-capped vials, combining with drug that Kira and Adja resuspended last week.

	Laurenobiolide (mg)	Desired conc (mg/mL)	volume methanol (uL)
Vial 1	1.2	8	150
Vial 2	2.3	8	287.5
	<b>total</b>		437.5

disc diffusion assays (20 ul/plate) 21.875

At this point, I do have enough to screen all six colonies. But if the first three are laurenobiolide-resistant, it seems wasteful to continue using up drug. Test three colonies and compare to WT and LB<sup>R</sup> controls.

Plan for tomorrow:

Plate	Bacteria	Drug	Control	Expectation
1	KRSA1	laurenobiolide (8 mg/mL)	methanol	LB sensitive
2	KRSA4	laurenobiolide (8 mg/mL)	methanol	LB resistant
3	KRSA1-PLBR-A	laurenobiolide (8 mg/mL)	methanol	?
4	KRSA1-PLBR-B	laurenobiolide (8 mg/mL)	methanol	?
5	KRSA1-PLBR-C	laurenobiolide (8 mg/mL)	methanol	?
6	KRSA1	Thiostrepton (50 mg/mL)	DMSO	TBD
7	KRSA4	Thiostrepton (50 mg/mL)	DMSO	TBD
8	KRSA1	fusidic acid (50 mg/mL)	EthOH	TBD
9	KRSA4	fusidic acid (50 mg/mL)	EthOH	TBD
10	KRSA1	Lincomycin (50 mg/mL)	water	TBD
11	KRSA4	Lincomycin (50 mg/mL)	water	TBD
12	KRSA1	Tiamulin (12.5 mg/mL)	EtOH	TBD
13	KRSA4	Tiamulin (12.5 mg/mL)	EtOH	TBD

Poured a flask of LB plates, 24 mL per plate, for the disc diffusion assays tomorrow.

**Sunday, July 30, 2023**

To Do:

1. Disc diffusion assay

## Results and Data:

Plan for today:

Plate	Bacteria	Drug	Control	Expectation
1	KRSA1	laurenobiolide (8 mg/mL)	methanol	LB sensitive
2	KRSA4	laurenobiolide (8 mg/mL)	methanol	LB resistant
3	KRSA1-PLBR-A	laurenobiolide (8 mg/mL)	methanol	?
4	KRSA1-PLBR-B	laurenobiolide (8 mg/mL)	methanol	?
5	KRSA1-PLBR-C	laurenobiolide (8 mg/mL)	methanol	?
6	KRSA1	Thiostrepton (50 mg/mL)	DMSO	TBD
7	KRSA4	Thiostrepton (50 mg/mL)	DMSO	TBD
8	KRSA1	fusidic acid (50 mg/mL)	EthOH	TBD
9	KRSA4	fusidic acid (50 mg/mL)	EthOH	TBD
10	KRSA1	Lincomycin (50 mg/mL)	water	TBD
11	KRSA4	Lincomycin (50 mg/mL)	water	TBD
12	KRSA1	Tiamulin (12.5 mg/mL)	EtOH	TBD
13	KRSA4	Tiamulin (12.5 mg/mL)	EtOH	TBD

Label DDA plates (13) with number of experiment, strain, drug, and date.

Scrape up cells of different strains into LB. Check OD600 (50 uL into 950). Too concentrated, dilute and check again.

Number	Strain	Measured OD600	Actual OD600	Desired OD600	Desired volume	Volume cells to add	Volume LB
1	KRSA1	0.143	2.86	0.05	1500	26.2	1473.8
2	KRSA4	0.148	2.96	0.05	1500	25.3	1474.7
3	KRSA1-PLBR-A	0.128	2.56	0.05	1500	29.3	1470.7
4	KRSA1-PLBR-B	0.181	3.62	0.05	1500	20.7	1479.3
5	KRSA1-PLBR-C	0.139	2.78	0.05	1500	27.0	1473.0

Dilute as in table above.

Plate 100 uL per plate, as per table, spreading with beads

Let plates dry.

While drying, prepare abx discs. Need 20 uL per disc- looks like we don't have >40 uL for the four trial abx. Dilute some of what is left to test.

	tiamulin	fusidic acid	thiostrepton	lincomycin
<b>Stock (mg/mL):</b>	12.5	50	50	50
<b>volume stock (ul)</b>	5	15	15	15
<b>volume dilutant (ul)</b>	45	45	45	45
<b>final conc (mg/ml)</b>	1.25	12.5	12.5	12.5
<b>dilutant</b>	EtOH	EtOH	DMSO	water

## Revised DDA sheet:

Plate	Bacteria	Drug	Control	Expectation
1	KRSA1	laurenobiolide (8 mg/mL)	methanol	LB sensitive
2	KRSA4	laurenobiolide (8 mg/mL)	methanol	LB resistant
3	KRSA1-PLBR-A	laurenobiolide (8 mg/mL)	methanol	?
4	KRSA1-PLBR-B	laurenobiolide (8 mg/mL)	methanol	?
5	KRSA1-PLBR-C	laurenobiolide (8 mg/mL)	methanol	?
6	KRSA1	thiostrepton (12.5 mg/mL)	DMSO	TBD
7	KRSA4	thiostrepton (12.5 mg/mL)	DMSO	TBD
8	KRSA1	fusidic acid (12.5 mg/mL)	EthOH	TBD
9	KRSA4	fusidic acid (12.5 mg/mL)	EthOH	TBD
10	KRSA1	lincomycin (12.5 mg/mL)	water	TBD
11	KRSA4	lincomycin (12.5 mg/mL)	water	TBD
12	KRSA1	tiamulin (1.25 mg/mL)	EtOH	TBD
13	KRSA4	tiamulin (1.25 mg/mL)	EtOH	TBD

In sterile petri dishes, add 20 ul of dilutant or drug to sterile discs. Let dry (methanol and ethanol, <10 minutes; DMSO >20 minutes!)

Plate discs on appropriate plates, incubate O/N at 37°C. Put in incubator around 2pm.

### Future To-Do

Check on plasmids that might restore growth to  $\Delta rpsU1\Delta rpsU2$  cells (Hannah's gDNA insert screen, Oli's Fall 2021 project).

Move 1° LVS pKR10-1 into strain box

## Bibliography

Suh, Moo-Jin et al. "Extending ribosomal protein identifications to unsequenced bacterial strains using matrix-assisted laser desorption/ionization mass spectrometry." *Proteomics* vol. 5,18 (2005): 4818-31. doi:10.1002/pmic.200402111