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February 2020**Thursday, February 20, 2020****To Do:**

1. ~~Streak out strains for mac expt~~
 - a. ~~LVS~~
 - b. ~~KRLVS1 *dptA*(-)~~
 - c. ~~KRLVS42 Δ *chaC*~~
2. ~~Seed macrophage~~
3. ~~Passage macrophage~~

Results and Data:**Streak out strains:**

All from original stocks, no single-use aliquots
 LVS, KRLVS1.1 (*dptA*(-)), KRLVS42.1 (Δ *chaC*)

Seed macrophage:

Remove old media, replace with 10 mL fresh media
 Scrape up all cells
 Spin 4' 4000 rpm in Howlett lab centrifuge
 Discard supernatant
 Resuspend cells in 7 mL DMEMF
 Check cell density using hemocytometer:

52	43	36	33	
55	27	42	16	
Average				38
Undiluted				76
Density				7.60E+05

Good density, make up enough cells for 14 mL at the correct density so I can use a multichannel.

$(17 \text{ mL})(16.7e4) / 76.0e4 = 3.1 \text{ mL plus } 10.9 \text{ mL DMEM}$

Using multichannel, transfer 150 uL to wells in 96-well plates, **2X**, as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	LVS	LVS	LVS		Δ <i>chaC</i>	Δ <i>chaC</i>	Δ <i>chaC</i>		<i>dptA</i> (-)	<i>dptA</i> (-)	<i>dptA</i> (-)	
B												
C												
D	LVS	LVS	LVS		Δ <i>chaC</i>	Δ <i>chaC</i>	Δ <i>chaC</i>		<i>dptA</i> (-)	<i>dptA</i> (-)	<i>dptA</i> (-)	
E												

Transfer 1 mL of undiluted cells with 9 mL DMEMF to new 100 mm TC plate.

Prepare cysteine (or mock) supplementation:

Cysteine Supplementation Calculations

Total final volume in well	0.2
Volume to plate (mL)	0.05
Total volume needed	4
Final conc cysteine in well (mM)	5
Stock cysteine (mM)	500
Conc cysteine to add (mM)	20
Volume stock cysteine (mL)	0.16
Volume media	3.84

Using multichannel, add 50uL mock to row A and 50uL cysteine to row D in both plates

Put 96-well plates and TC plate in Dutta lab CO2 incubator.

Check density of cells plated:

2nd measurement			
8	14	6	7
12	6	6	2
19	10	20	1
5	5	9	8
Average			8.625
Undiluted			17.25
Density			1.73E+05

A good density, very close to what I was aiming for, 2.59×10^4 rather than 2.5×10^4 .

Friday, February 21, 2020

To Do:

1. Prepare inoculum
2. Infect macro
3. Wash macro
4. T=2

Results and Data:

Plan for inoculums:

Bacterial Calculations	Actual
MOI	5
Macrophage cells per well	2.59×10^4
Volume bacteria to add (mL)	0.05
Bacterial density needed (cells/mL)	2.59×10^6
Cells/mL per OD600	5.81×10^9
OD needed for given density	0.00045
Resuspend to	0.045
Final MOI 5, dilute 1:100	0.00045

Prepare 96-well plates with 1x PBS for dilution of inoculums and additional dilution.

Scrape up cells into 300 μ L DMEMF, resuspend, add 650 μ L more DMEMF, check OD600 with 1:10 dilution. Calculate the volume of resuspended cells to add to new tube of DMEMF to get 1.3 mL of 0.045. Use 1mL to check OD600. Calculate the volume of diluted cells to add to 5 mL DMEMF to get a final OD600 of 0.0004.

Number	Strain	Resuspend cells to (OD600)	For final vol 1.3 mL at 0.045		OD600	Volume to dilute in 1 mL (usually 1:100)	Volume PBS or cysteine	Volume media removed from 5mL
			Cells (μ L)	Media ($\times 2$, μ L)				
1	LVS	1.61	36.3	963.7	0.042	53.00	50	103.0
2	Δ chaC	1.25	46.8	953.2	0.042	53.00	50	103.0
3	dptA(-)	1.58	37.0	963.0	0.043	53.00	50	103.0

Do 2x: once in media with mock, once in +cysteine media

Dilute cells for inoculum in 50 mL conicals- have 6 tubes, representing 3 strains +/- cysteine.

#		Cysteine?
1	LVS	-
2	Δ chaC	-
3	dptA(-)	-
4	LVS	+
5	Δ chaC	+
6	dptA(-)	+

Bring inoculum, 96-well plates, and other necessary items to hood upstairs.

Pour inoculums from 50 mL conical into 3-well reservoir, 3 at a time. Use multichannel (2-50) to add 50uL cells to each well as indicated in plate setup. Transfer 200uL each inoculum to 96-well plate for serial dilutions in PBS.

Put 96-well plate in 37°C CO₂ incubator in Camberg lab TC room (has water in bottom).

Infection done at 10:30AM.

Serially dilute inoculums (1 → 10⁻⁴) and plate on square CHA plates using drip method. Put square plates at 37°C.

Wash at 12:30PM

Have prepared 7mL DMEMF + gentamycin +/- cysteine.

Cysteine

Total volume needed (uL)	7000
Final conc cysteine (mM)	5
Stock cysteine (mM)	500
Volume stock cysteine (uL)	70

Gentamycin

Gentamycin media (uL)	7000
Stock gentamycin (ug/mL)	50000
Desired concentration (ug/mL)	10
Volume gentamycin (uL)	1.4

Pipetted 7 mL DMEMF into 50mL conicals

Removed 70uL DMEMF

Added cysteine or PBS mock

Added 1.4uL gentamycin

Remove media from all wells

Add 280uL 1x PBS to all wells (wash 1)

Remove PBS

Add 280uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL DMEMF + gentamycin +/- cysteine to all wells as indicated in plate setup.

Put 96-well plate in 37°C CO₂ incubator in Camberg lab TC room (has water in bottom).

T=2 at 2:30pm

Have labeled round CHA plates and brought up single-use spreaders for spreading.

Remove 1 infected 96-well macrophage plate from incubator.

Remove media from all wells

Add 250uL 1x PBS to all wells (wash 1)

Remove PBS

Add 250uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL 1% saponin in 1x PBS to all wells

Let set at room temp for 30 mins

Plate 50uL each well on a round plate, 2x.

Put plates at 37°C.

Saturday, February 22, 2019**To Do:**

1. T=24

Results and Data:**T=24 at 12:30pm**

Have labeled square and round CHA plates and added glass beads for spreading to round plates.

Have one 96 well plates prepped with 1X PBS for serial dilutions.

Remove last infected 96-well macrophage plate from incubator.

Remove media from all wells

Add 250uL 1x PBS to all wells (wash 1)

Remove PBS

Add 250uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL 1% saponin in 1x PBS to all wells

Let set at room temp for 30 mins

Transfer 180 uL of row A1-A3, D1-D3, D9-D11 to plate with PBS for serial dilutions.

For row A5-7, A9-A11, D5-D7, plate 50uL each well on a round plate, 2x.

Plate serial dilutions 2x on square plates.

Put plates at 37°C.

Sunday, February 23, 2020**To Do:**

1. Count inoculum plates

Results and Data:**Plate counts**

Inoculum												
	Cysteine?	Replicate	3	4	Dilution factor counted	Cells / mL	Average Cells / mL	St dev	CFU/well	St dev	MOI (based on number of seeded macrophage-see setup)	
LVS	-	1A	TMTC	35	0.001	3.50E+06	3.15E+06	4.95E+05	1.58E+05	2.47E+04	6.1	
	-	1B	TMTC	28	0.001	2.80E+06						
Δ chaC	-	2A	TMTC	36	0.001	3.60E+06	3.10E+06	7.07E+05	1.55E+05	3.54E+04	6.0	
	-	2B	TMTC	26	0.001	2.60E+06						
dptA(-)	-	3A	TMTC	34	0.001	3.40E+06	3.45E+06	7.07E+04	1.73E+05	3.54E+03	6.7	
	-	3B	TMTC	35	0.001	3.50E+06						
LVS	+	7A	TMTC	33	0.001	3.30E+06	3.50E+06	2.83E+05	1.75E+05	1.41E+04	6.8	
	+	7B	TMTC	37	0.001	3.70E+06						
Δ chaC	+	8A	TMTC	28	0.001	2.80E+06	2.80E+06	1.41E+05	1.40E+05	7.07E+03	5.4	
	+	8B	TMTC	26	0.001	2.60E+06						
dptA(-)	+	9A	TMTC	35	0.001	3.50E+06	4.05E+06	7.78E+05	2.03E+05	3.89E+04	7.8	
	+	9B	TMTC	46	0.001	4.60E+06						
Dilution Factor			0.01	0.001								

MOIs look good and consistent.

Monday, February 24, 2020

To Do:

- Count T=2 plates, T=24 plates

Results and Data:

Plate counts

	Cysteine?	Plate	Plate 1	Plate 2	Dilution factor counted	Average Cells	CFU per well	T-test
LVS		1A	6	4	1	5	2.00E+01	#DIV/0!
		1B	6	4	1	5	2.00E+01	
		1C	4	6	1	5	2.00E+01	
Δ chaC		2A	7	4	1	5.5	2.20E+01	0.187
		2B	1	3	1	2	8.00E+00	
		2C	1	1	1	1	4.00E+00	
dptA(-)		3A	10	3	1	6.5	2.60E+01	0.281
		3B	11	9	1	10	4.00E+01	
		3C	5	4	1	4.5	1.80E+01	
LVS		7A	5	3	1	4	1.60E+01	1.000
		30	9	3	1	6	2.40E+01	
		7C	5	5	1	5	2.00E+01	
Δ chaC		8A	5	5	1	5	2.00E+01	0.124
		8B	3	4	1	3.5	1.40E+01	
		8C	1	5	1	3	1.20E+01	
dptA(-)		9A	16	5	1	11	4.20E+01	1.000
		9B	1	7	1	4	1.60E+01	
		9C	2	5	1	4	1.40E+01	

50 ul cells plated

Great, all the macrophage were infected and to similar extents!

T=2							
#		Cysteine?	Average CFU per well	St Dev	Original MOI	T-test	Fold Change
1	LVS	-	2.00E+01	0.00E+00	6.1		1.00
2	Δ chaC	-	1.13E+01	9.45E+00	6.0	0.187	-1.76
3	dptA(-)	-	2.80E+01	1.11E+01	6.7	0.281	1.40
4	LVS	+	2.00E+01	4.00E+00	6.8	1.000	1.00
5	Δ chaC	+	1.53E+01	4.16E+00	5.4	0.124	-1.30
6	dptA(-)	+	2.40E+01	1.56E+01	7.8	0.680	1.20

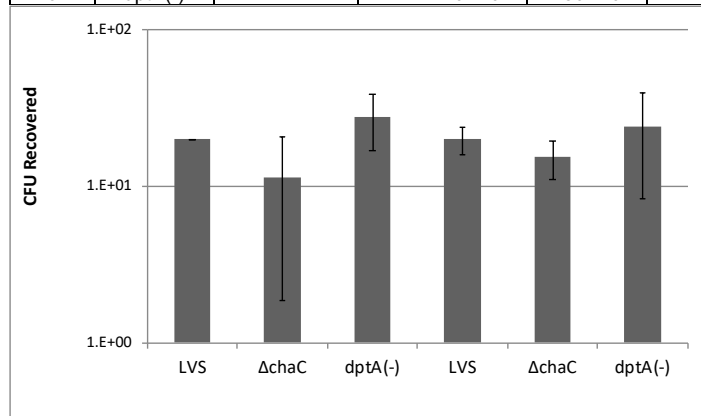
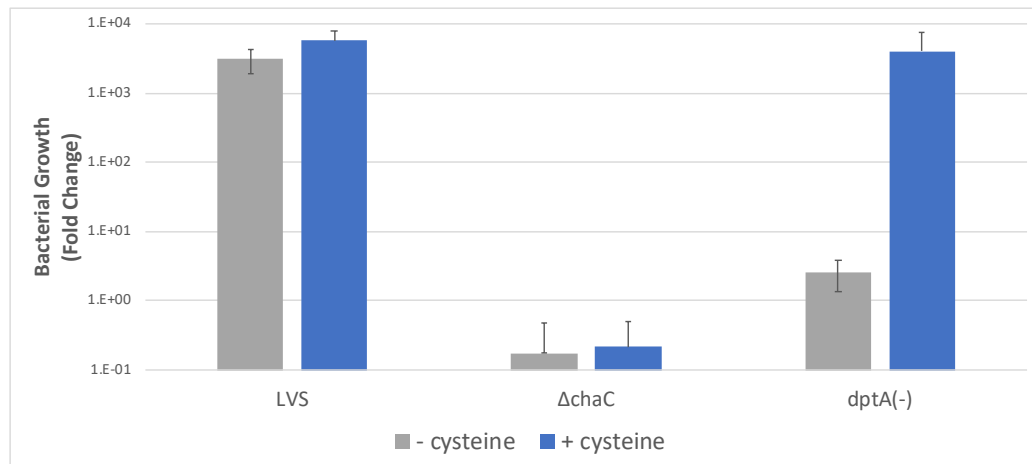


	Plate	Track Plate 1			Track Plate 2			Dilution factor counted	Average Cells	CFU per well
		1	2	3	1	2	3			
LVS	1A	TMTC	TMTC	26	TMTC	TMTC	23	0.01	25	4.90E+04
	1B	TMTC	TMTC	20	TMTC	TMTC	29	0.01	25	4.90E+04
	1C	TMTC	TMTC	43	TMTC	TMTC	46	0.01	45	8.90E+04
ΔchaC*	2A	1			0			1	1	2.00E+00
	2B	1			1			1	1	4.00E+00
	2C	0			0			1	0	0.00E+00
dptA(-)*	3A	25			24			1	24.5	9.80E+01
	3B	28			40			2	34	6.80E+01
	3C	50			30			3	40	5.33E+01
LVS	7A	TMTC	TMTC	60	TMTC	TMTC	56	0.01	58	1.16E+05
	7B	TMTC	TMTC	70	TMTC	TMTC	81	0.01	76	1.51E+05
	7C	TMTC	TMTC	34	TMTC	TMTC	43	0.01	39	7.70E+04
ΔchaC*	8A	3			1			1	2	8.00E+00
	8B	0			0			1	0	0.00E+00
	8C	1			0			1	0.5	2.00E+00
dptA(-)	9A	TMTC	TMTC	47	TMTC	TMTC	58	0.01	53	1.05E+05
	9B	TMTC	TMTC	81	TMTC	TMTC	55	0.01	68	1.36E+05
	9C	TMTC	TMTC	27	TMTC	TMTC	22	0.01	25	4.90E+04
Dilution Factor		1	0.1	0.01	1	0.1	0.01			

*Plated 50 ul on circular plate

T=24

	Cysteine?	Average CFU per well	St Dev	Original MOI	T-test (vs LVS)	Fold Change
LVS	-	6.23E+04	2.31E+04	6.1		1.00
ΔchaC	-	2.00E+00	2.00E+00	6.0	0.009	-31166.67
dptA(-)	-	7.31E+01	2.28E+01	6.7	0.010	-852.58
LVS	+	1.15E+05	3.70E+04	6.8	0.106	1.84
ΔchaC	+	3.33E+00	4.16E+00	5.4	0.009	-18700.00
dptA(-)	+	9.67E+04	4.41E+04	7.8	0.298	1.55



Confirmed final round plate numbers on 2/25.

Future To-Do

Move 1° LVS pKR10-1 into strain box

Bibliography