Dunaliella Salina vs. Halobacterium

Purpose

Prior studies and photos have suggested that *Dunaliella Salina* and *Halobacterium* may exist symbiotically. We set out to validate other studies by testing whether *Dunaliella Salina* and *Halobacterium* grow faster in the presence of each other.

Materials for Set up

- *Halobacterium* (Great Salt Lake)
- *Dunaliella Salina* 19/18
- 12 30 mL Bottles
- 750 µL 5% Case Amino Acids
- 310 mL 3.5 M Dunaliella Medium
- Micropipette: 200 µL, 1000 µL
- Pipette: 25 mL
- Eppendorf Centrifuge
- Tips
- Serological Syringe
- C-Chip
- Clicker
- Microscope: 40x total
- OD Machine
- OD Chambers

Materials for Population Calculation (per day)

- 20 mL 3.5 M Dunaliella Medium
- 60 µL 1% Formaldehyde
- 4 Complex Medium Plates
- 126 1 mL Tubes
- Vortex
- Micropipette: 20 µL, 200 µL, 1000 µL
- Tips
- C-Chip
- Clicker
- Microscope: 40x total
- OD Machine
- OD Chambers
- Freezer
- Eppendorf Centrifuge

Cultures you should have

A. *Dunaliella Salina A*
B. *Dunaliella Salina B*
C. *Dunaliella Salina C*
D. *Halobacterium + Amino Acids A*
E. *Dunaliella Salina + Halobacterium A*
F. *Dunaliella Salina + Halobacterium B*
G. *Dunaliella Salina + Halobacterium C*
H. *Halobacterium + Amino Acids B*
I. *Halobacterium A*
J. *Halobacterium B*
K. *Halobacterium C*
L. *Halobacterium + Amino Acids C*

Note: For the second experiment, we inoculated a *Dunaliella salina + Halobacterium + Amino Acids* group of cultures, and used four replicates instead of three. In order to truly hold all controls, you should also create a group of cultures of *Dunaliella salina + Amino Acids.*

Preparation

1. Obtain 1 mL of *Halobacterium*
2. Remove the Amino Acids by centrifuging the solution and removing the liquid
3. Resuspend the solid with 1 mL of fresh 3.5 M MM_3
4. Repeat steps 2 and 3 two times
5. Move the solution to a new tube and mix in 150 µL of MM_3
6. Add 150 µL of the sample to a OD chamber and estimate the density via machine
7. Count the density of the Dunaliella with a C-Chip
8. Determine the amount to add of *Dunaliella Salina* and *Halobacterium* to have a ratio of $2.86*10^6$ *D. Salina* to $2.64*10^8$ *Halobacterium.* (In our case, 400 µL of *D. Salina* and 100 µL of *Halobacterium*)
9. Add 25 mL to 12 bottles
10. Add *D. Salina* at the determined volume to 6 bottles.
11. Add *Halobacterium* at the determined volume to 3 bottles with *D. Salina* in them, and 6 other bottles
12. Add 250 µL of 5% Case Amino Acids to 3 bottles with *Halobacterium* in them

Population Calculation Procedure (Done MWF)

Estimating *Dunaliella Salina* density (Cultures: *Dunaliella Salina, Dunaliella Salina* + *Halobacterium*)

1. Prepare six tubes with 10µL of 1% Formaldehyde.
2. Add 90 µL of one culture per tube
3. Vortex the tubes
4. For each tube, add 10 µL to one side of a C-Chip
5. Count all the Dunaliella in the four corner squares using a clicker and resetting after each square
6. Calculation: $Y= \frac{(X_1+X_2+X_3+X_4)}{4} \cdot \frac{10}{9} \cdot \frac{10^4}{1}$

Note: For the second trial, in order to get more accurate counts, we counted ten big squares' worth of cells.

Estimating *Halobacterium* density (Cultures: *Halobacterium* + Amino Acid*s, Halobacterium*)

1. For each culture, add 150 µL to an OD chamber
2. Add 150 µL of distilled water to an OD chamber for your blank
3. Measure them with an Optical Density machine

Note: For the second trial, we used a SpectraMax M5 to obtain more accurate data and make organization easier. We measured Absorbance at wavelengths of 600, 647, and 664 and Fluorescence at an excitation of 488 and an emission of 685. We used a 96 well plate and loaded 2 wells of 200 µL of each sample.

Estimating *Halobacterium* density (Cultures: All)

1. Add 450 µL of 3.5 M Dunaliella medium to 12 tubes
2. Add 180 µL of 3.5 M Dunaliella medium to 72 tubes
3. Add 50 µL of *Dunaliella Salina A* to a tube containing 450 µL of medium (A1)
4. Vortex tube A1
5. Add 20 µL of A1 to a tube containing 180 µL of medium (A2)
6. Vortex tube A2
7. Repeat steps 5 and 6 until you have a tube diluted in *Dunaliella Salina A* content by 10^{-7} (A7)
8. Repeat steps 3 to 7 for each culture
9. Spot 20 µL of solutions diluted by 10^{3} to 10^{7}. Repeat 4 times.
10. Count the number of colonies under a microscope after a week. Count one spot from each replicate. Note the dilution of each spot counted.
11. Calculate the estimate cell density from each spot: $\frac{x}{20} \cdot \frac{100^{\text{Dilution}}}{10^{\text{Dilution}}} \cdot \frac{1000}{1}$

Note: For the second trial, we sterilized the micropipette used for collecting the sample in between different types of cultures. We used 96 well plates to hold the samples and a multipipette to add the buffer and dilute the samples. We used 180 µL of medium and 20 µL of culture for the initial dilution. We also used dilutions 10^{-1} to 10^{-5} to spot the plate and switched to 10 µL of sample for spotting (so the x, the number of cells, would be divided by 10, not 20).

Estimating *Dunaliella Salina* and *Halobacterium* Density (Cultures: All)

1. For each culture, make 3 tubes containing 100 µL of the sample
2. Centrifuge them for 1 minute at 16.1 rcf.
3. Store them in the freezer
4. Perform qPCR

Note: We lack the information for this trial, as Nicolas Pinel performed it after we left ISB.

Trial 1:

Dunaliella Hemocytometer Cell Counts

	13-Jul	18-Jul	20-Jul	22-Jul	25-Jul	27-Jul	29-Jul*	1-Aug**
Duna A	114000	578000	889000	1650000	1670000	1430000	11300000	1375000
Duna B	114000	614000	1050000	1440000	1250000	500000	13300000	1187500
Duna C	114000	528000	1040000	1590000	1190000	867000	7880000	1437500
Hbt+Duna A	114000	722000	964000	1700000	2380000	5980000	34400000	2787500
Hbt+Duna B	114000	514000	781000	1460000	1480000	1540000	12400000	1687500
Hbt+Duna C	114000	536000	608000	1650000	1720000	3190000	15300000	1275000

Halobacterium Optical Density Measurements

	13-Jul	18-Jul	20-Jul	22-Jul	25-Jul	27-Jul	29-Jul	1-Aug
Hbt+AA A	0	0.047	0.2075	0.2321	0.3171	0.1846	0.1915	0.2581
Hbt+AA B	0	0.0468	0.2144	0.2141	0.2841	0.1813	0.2023	0.2546
Hbt+AA C	0	0.05	0.1781	0.1574	0.2035	0.1287	0.1948	0.2287
Hbt A	0	0.004	0	0	0.011	-0.007	0.001	0.0145
Hbt B	0	0.01	0.003	-0.016	0.003	-0.008	0	0.001
Hbt C	0	0.002	0.0108	0	0	-0.006	0	0

Halobacterium Spotting Cell Counts

	13-Jul	18-Jul	20-Jul	22-Jul	25-Jul	27-Jul	29-Jul
Duna A	0	0	0	0	0	0	0
Duna B	0	0	0	0	0	0	0
Duna C	0	0	0	0	0	0	0
Hbt+Duna A	1200000	1550000	1330000	450000	125000	0	125000
Hbt+Duna B	1200000	1090000	1310000	188000	12500	25000	0
Hbt+Duna C	1200000	600000	588000	0	0	0	0
Hbt A	1200000	1210000	1390000	0	1880000	988000	1875000
Hbt B	1200000	1710000	925000	9630000	1100000	825000	1100000
Hbt C	1200000	1430000	888000	188000	875000	1750000	875000
Hbt+AA A	1200000	40000000	47500000	58800000	70000000	72500000	70000000
Hbt+AA B	1200000	13500000	53800000	0	51300000	80000000	51250000
Hbt+AA C	1200000	17100000	55000000	55000000	53800000	58800000	53750000

Trial 2:

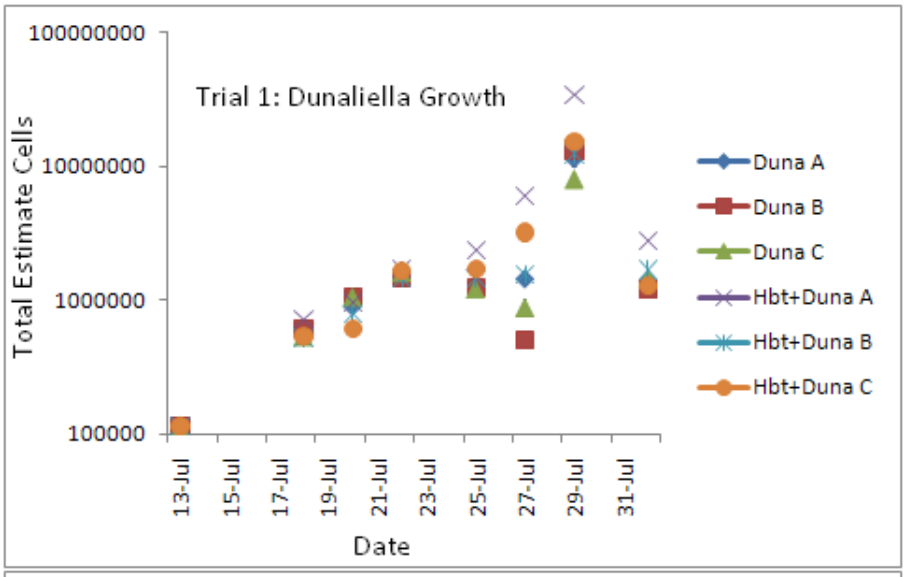
Averaged Halobacterium Spotting Counts

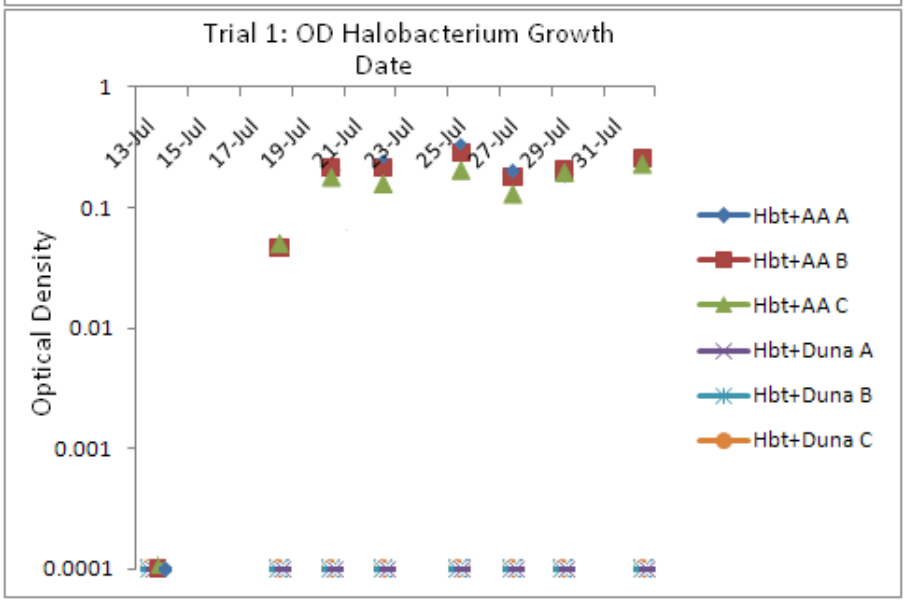
	8-Aug	10-Aug	12-Aug	14-Aug	15-Aug	16-Aug	17-Aug	19-Aug
Dun	0	0	0	0	0	0	0	0
DunHbt	606250	182500	443125	391250	176250	250094	169724	186938
DunHbtAA	562500	2484375	10031250	56593750	55204545	76590909	55020833	63781250
Hbt	875000	500000	784375	412000	304062	260000	273438	171250
HbtAA	5000000	5000000	12031250	72593750	87843750	72770833	52750000	58343750

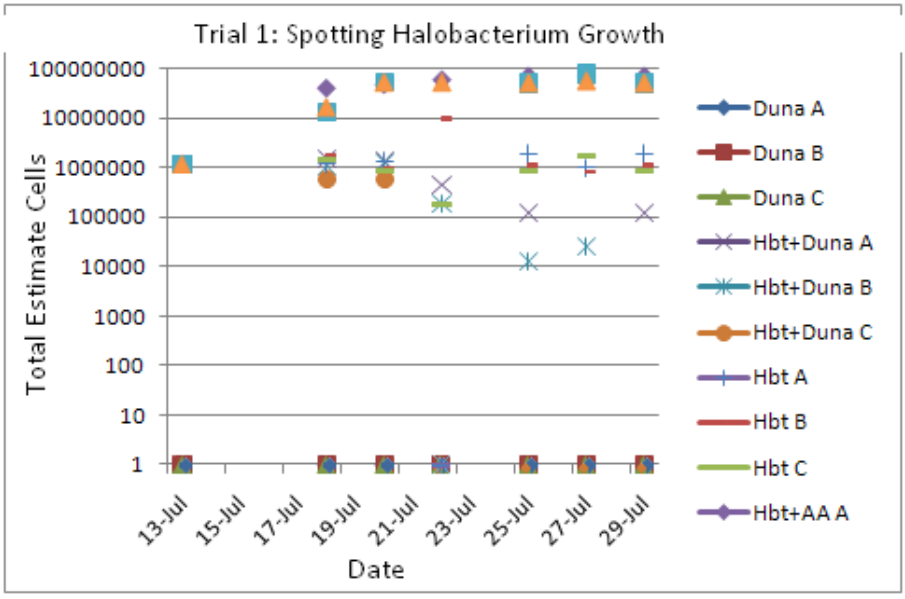
Averaged Dunaliella Counts

	12-Aug	16-Aug	19-Aug	22-Aug
Dun	1.10E+23	2.18E+23	4.07E+23	3.92E+23
DunHbt	1.33E+23	3.68E+23	1.02E+28	1.22E+31
DunHbtAA	1.52E+23	6.84E+23	1.38E+31	1.29E+31

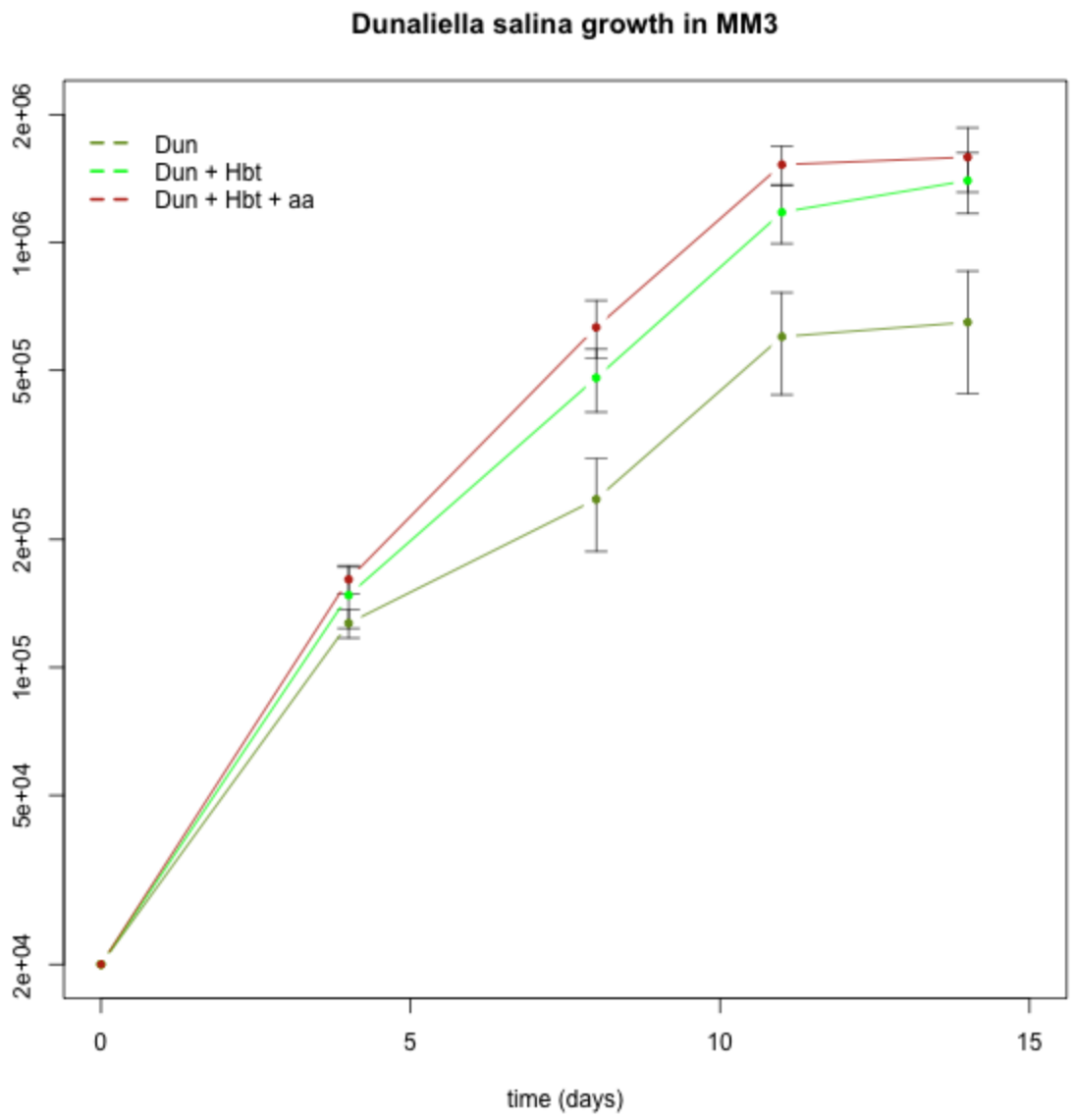
Trial 1:

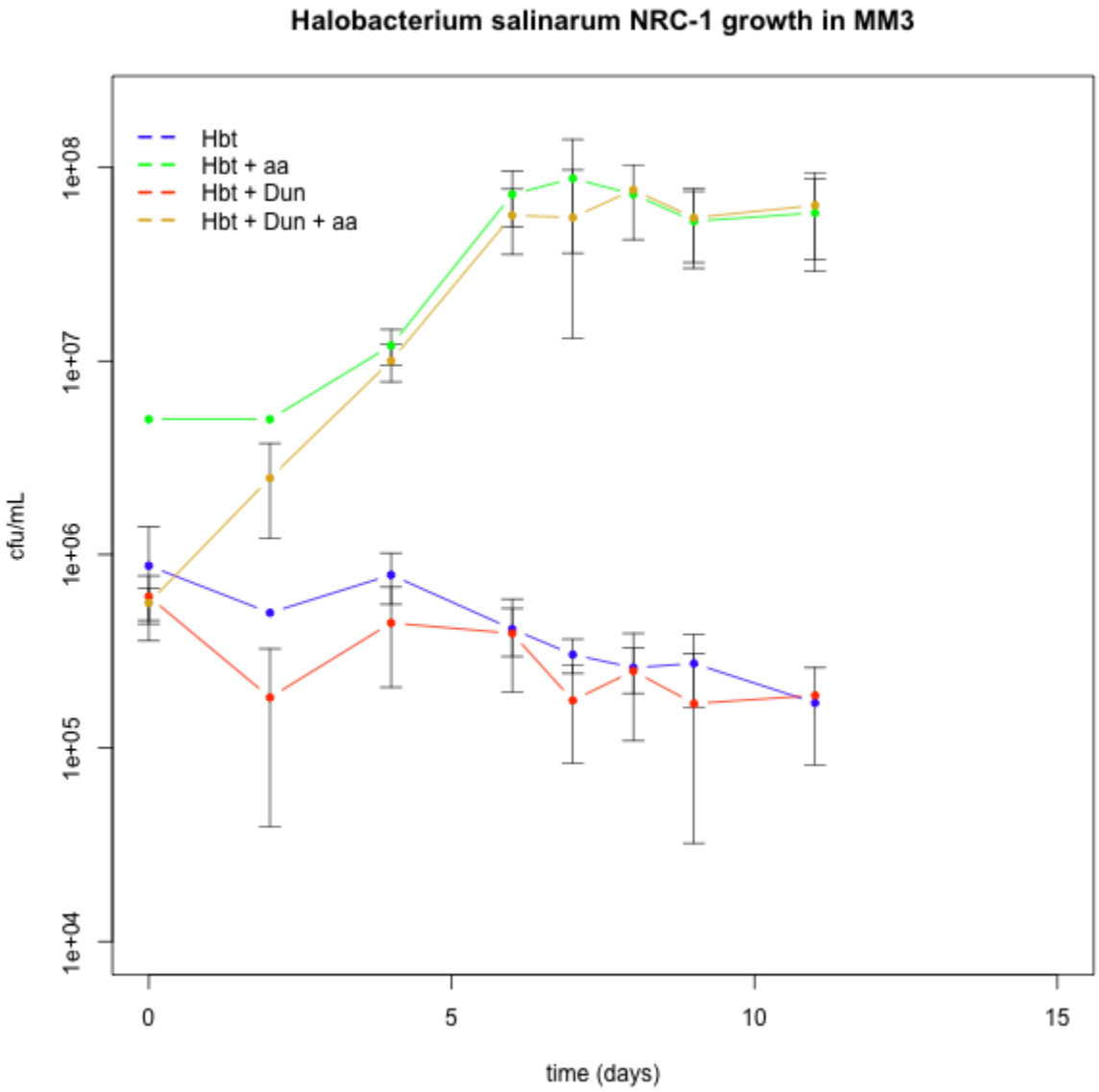

Trial 1: Dunaliella Growth
Total Estimate Cells
100000000
10000000
1000000
100000
13-Jul
15-Jul
17-Jul
19-Jul
21-Jul
23-Jul
25-Jul
27-Jul
29-Jul
31-Jul
Date
Duna A
Duna B
Duna C
Hbt+Duna A
Hbt+Duna B
Hbt+Duna C


Trial 1: OD Halobacterium Growth
Date
Optical Density
1
0.1
0.01
0.001
0.0001
13-Jul
15-Jul
17-Jul
19-Jul
21-Jul
23-Jul
25-Jul
27-Jul
29-Jul
31-Jul
Hbt+AA A
Hbt+AA B
Hbt+AA C
Hbt+Duna A
Hbt+Duna B
Hbt+Duna C


Trial 1: Spotting Halobacterium Growth
Total Estimate Cells
100000000
10000000
1000000
100000
10000
1000
100
10
1
13-Jul
15-Jul
17-Jul
19-Jul
21-Jul
23-Jul
25-Jul
27-Jul
29-Jul
Date
Duna A
Duna B
Duna C
Hbt+Duna A
Hbt+Duna B
Hbt+Duna C
Hbt A
Hbt B
Hbt C
Hbt+AA A

Trail 2:


Dunaliella salina growth in MM3
Dun
Dun + Hbt
Dun + Hbt + aa
2e+06
1e+06
5e+05
2e+05
1e+05
5e+04
2e+04
0
5
10
15
time (days)



Conclusions

Dunaliella salina seems to benefit from having *Halobacterium salinarum* present, as the *D. salina* cultures with *H. salinarum* grew to noticeably higher population. However, *D. salina*'s growth rate does not seem to change with the differing population levels of *H. salinarum*. Although our first trial supported the hypothesis that *D. salina* harm *H. salinarum*, the second trial shows that *H. salinarum* grows at the same rate with or without *D. salina*. *Dunaliella salina* and *Halobacterium salinarum* do not seem to be mutually beneficial to each other. Further trials, studying the effect of Amino Acids on *D. salina* as well, would help pinpoint *H. salinarum*'s role in encouraging *D. salina* growth.