The Effects of Low-Shear Simulated Microgravity on Soil Bacterium *Pantoea agglomerans*

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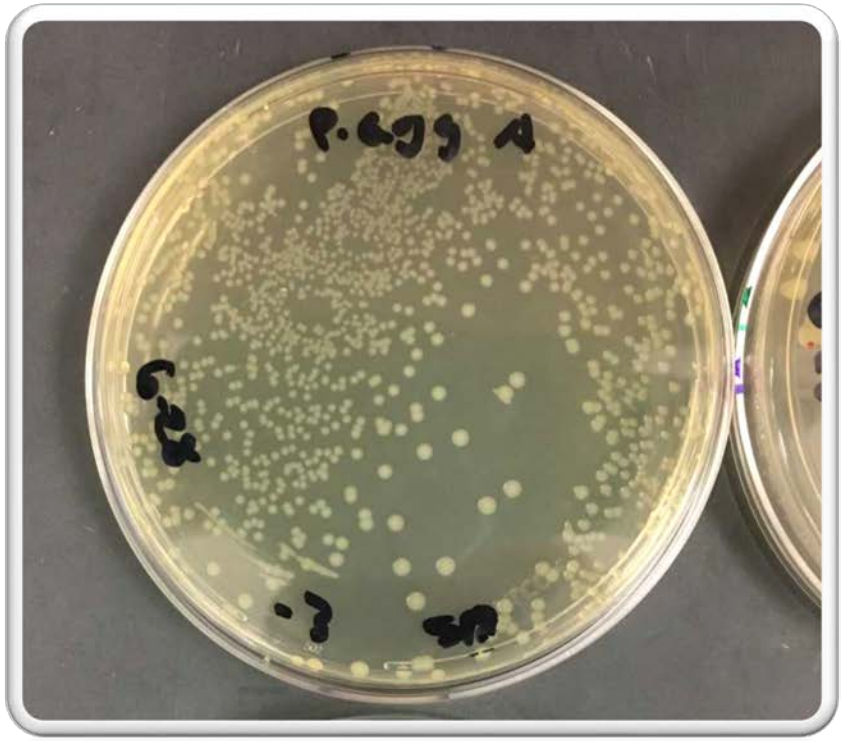
Objectives

- Determine growth rate of *Pantoea agglomerans* in simulated microgravity and normal gravity conditions.
- Determine antibiotic susceptibility of Pantoea *agglomerans* after exposure to simulated microgravity and normal gravity conditions.
- Have Fun ☺

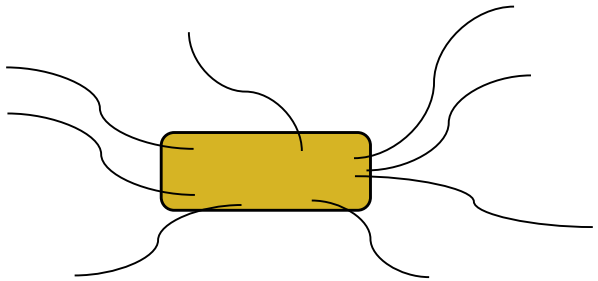
Materials

- Rotary Cell Culture System (RCCS)
- High Aspect-Ratio Vessel (HARV)
- Strain of *Pantoea agglomerans (ISS Isolate)*
- Spectrophotometer
- Trypticase Soy Broth (TSB) and Agar (TSA) Plates
- Mueller-Hinton Agar Plates
- Antibiotic Infused Disks
- GraphPad Prism (Statistical Analysis)
- High Spirit and a Good Attitude ☺

Panotea agglomerans



- *P. agglomerans*
- Gram-negative
- Rod-Shaped
- Motile – peritrichous
- Enterobacteriaceae Family (*E. coli, Klebsiella, Salmonella, Shigella, and Yersinia pestis)*



Low- Shear Simulated Microgravity (LSSMG)

- RCCS
- HARV
- Low Shear – no bubbles in vessel once filled
- Constant rotation rate
- Vertical Rotation (Fig.1) = Simulated Microgravity
- Horizontal No Rotation (Fig.2) = Normal Gravity

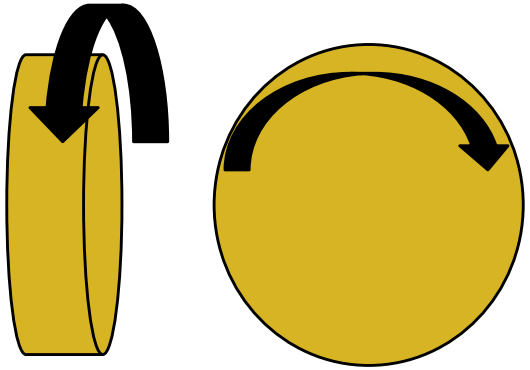


Fig.1

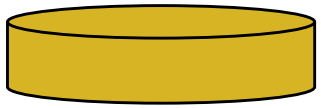
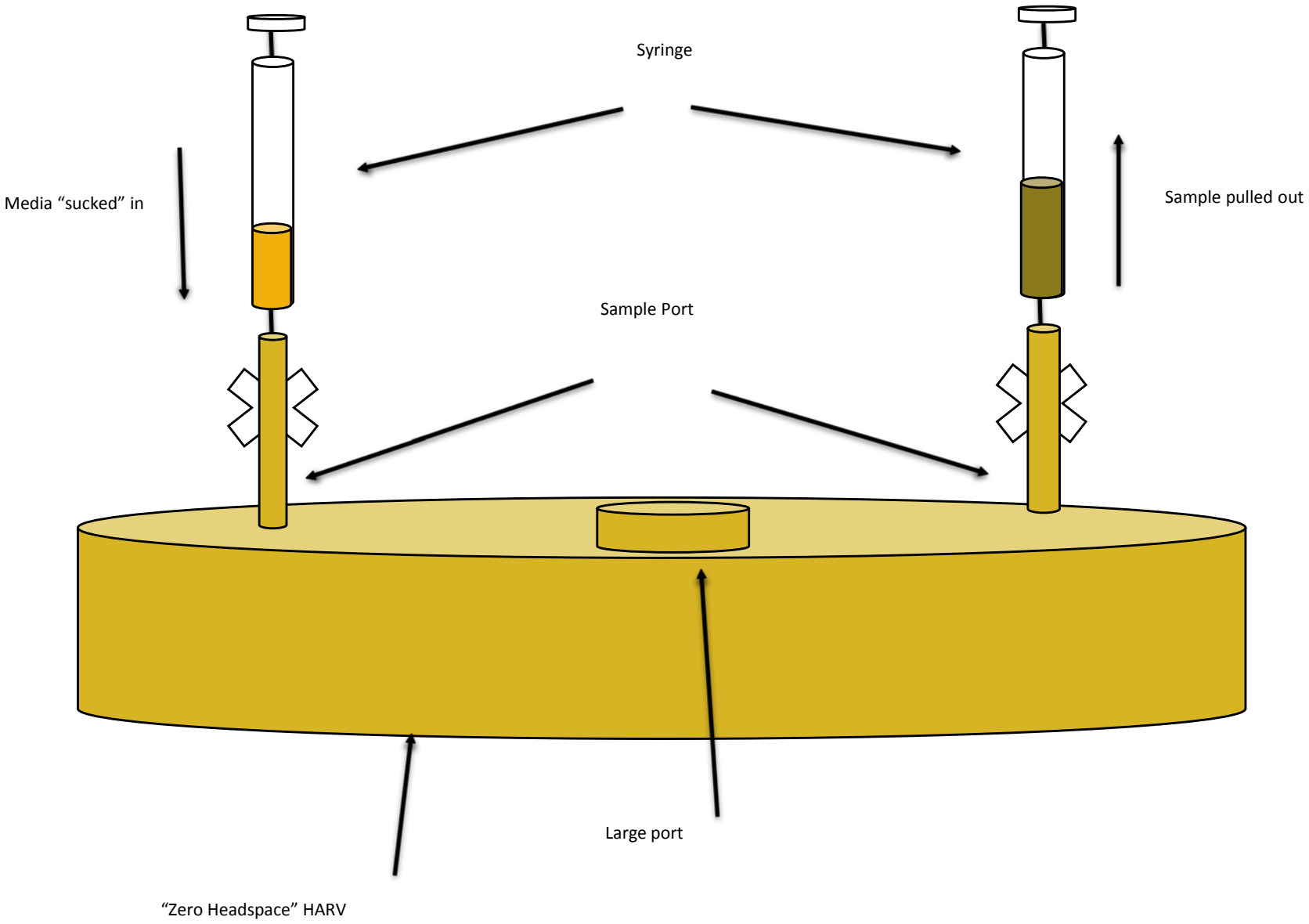


Fig. 2


Syringe
Media “sucked” in
Sample pulled out
Sample Port
Large port
“Zero Headspace” HARV

Relevance

- Plant Growth Promoter (Dutkiewicz et. al; Mirsha et. Al)
- AND Plant Pathogen (Cruz et. al, Dutkiewicz et. al)
- Opportunistic Pathogen (Büyükcama et. al, Cruz et. al)
- Immunosuppressed Astronauts (Mann et. al)
- Microbial growth in microgravity (Nickerson et. al)

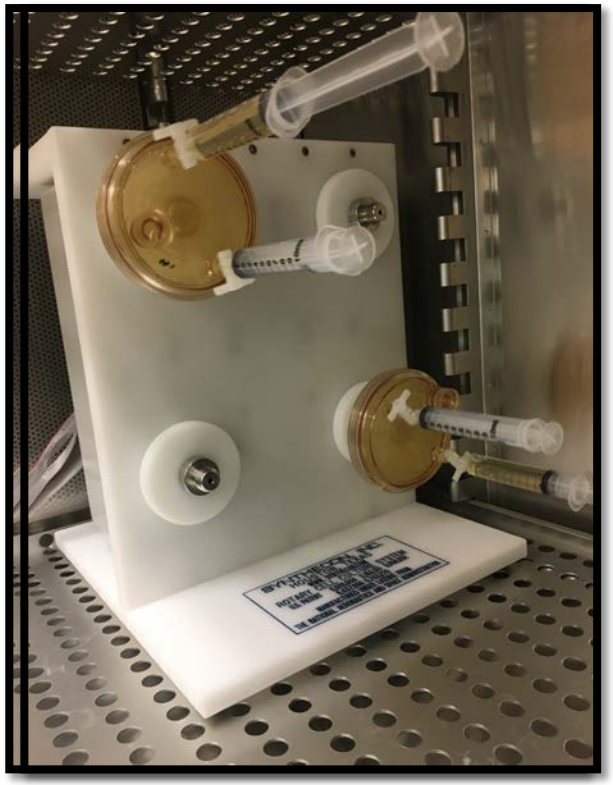
Hypotheses

- H_{1A} – *P. agglomerans* growth rate will increase in LSSMG compared to normal gravity.
- H_{2A} - *P. agglomerans* growth rate will decrease in LSSMG compared to normal gravity.
- H_{0A} – There will be no change in the growth rate of *P. agglomerans* in LSSMG compared to normal gravity.

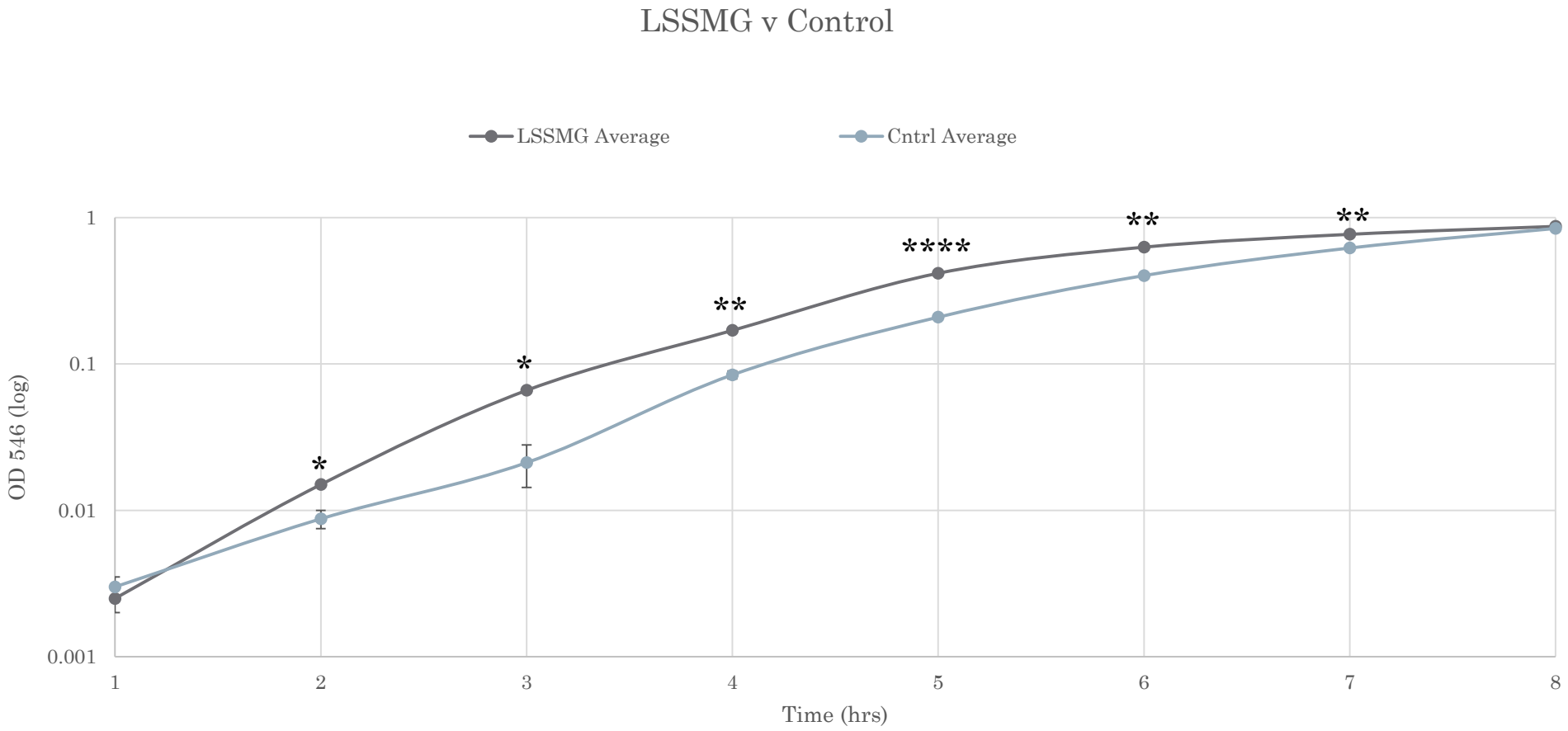
- H_{1B} – *P. agglomerans* will become less susceptible to chosen antibiotics after exposure LSSMG compared to normal gravity.
- H_{0B} – *P. agglomerans* will remain susceptible to chosen antibiotics after exposure to LSSMG compared to normal gravity.

Experimental Design – Exp. 1

- Determine growth rate in a shaking flask
- Determine growth rate in LSSMG and normal gravity
  - Experimental Rotated HARVS vertically
  - Horizontal Control HARVS
  - Shaking flask as ground control
  - Removed sample
  - Read optical density (OD)
  - Plated to confirm starting concentration



Exp. 1 Trial 1


LSSMG v Control
LSSMG Average
Cntrl Average
OD 546 (log)
1
0.1
0.01
0.001
1
2
3
4
5
6
7
8
Time (hrs)
*
*
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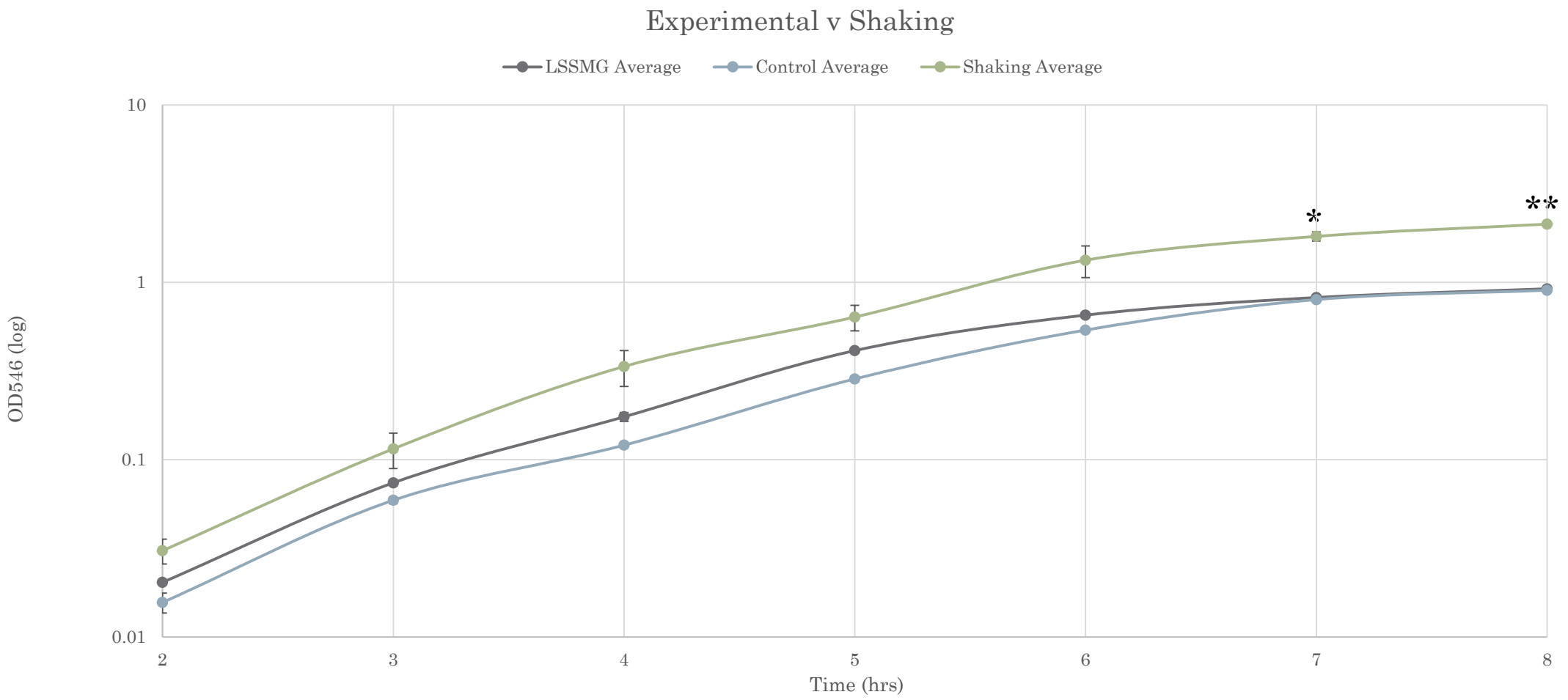
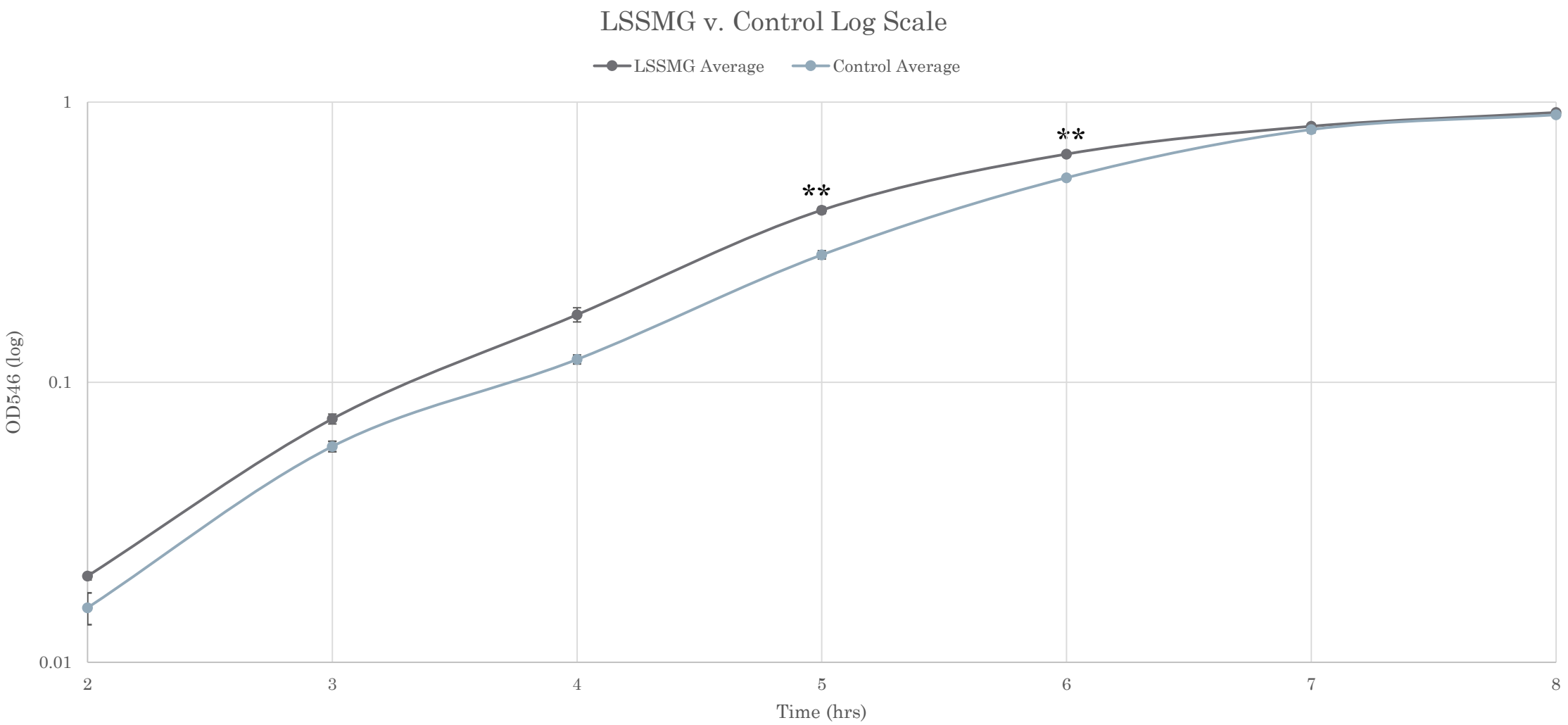
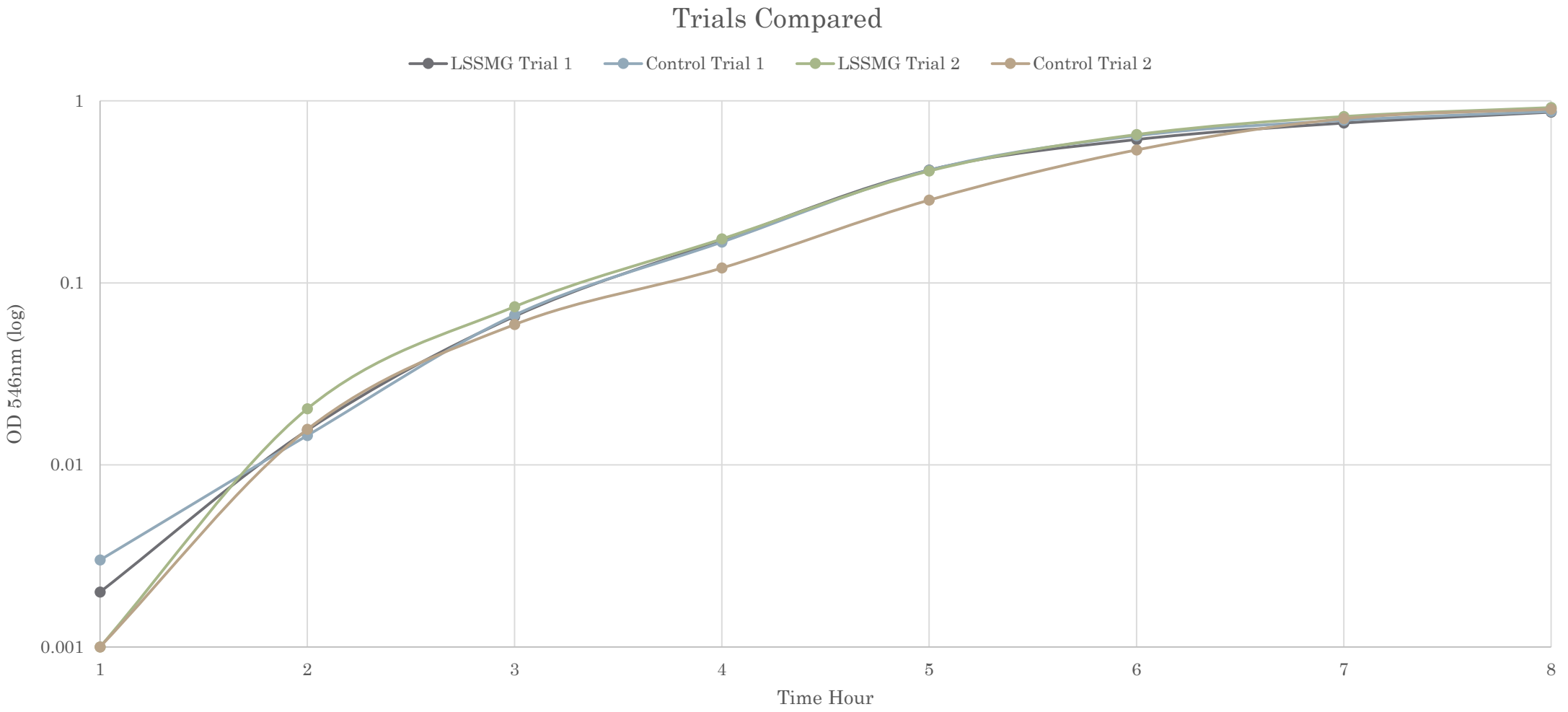
Exp. 1 Trial 2 Results



Exp. 1 Trial 2 Results – Cont.

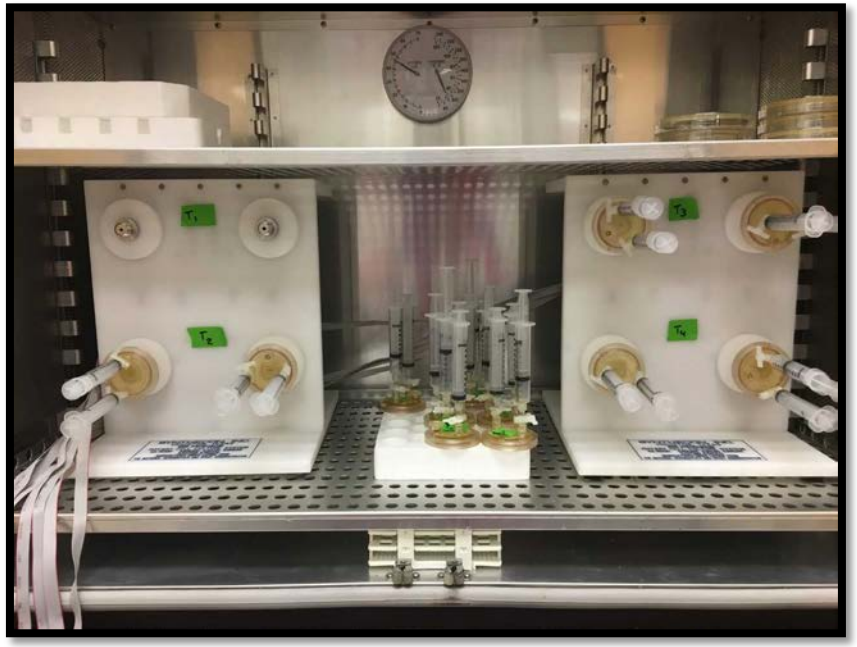


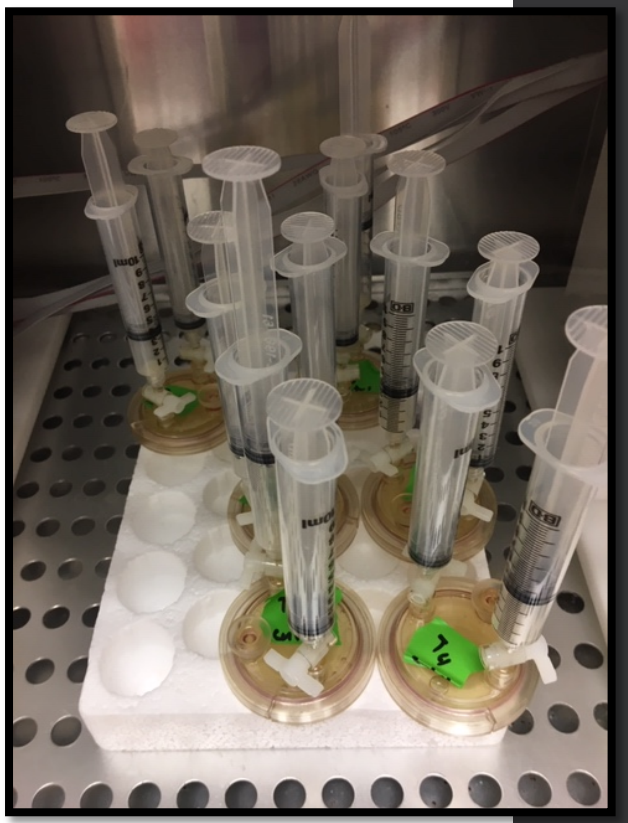
Exp. 1 Trial 1 & 2 Compared



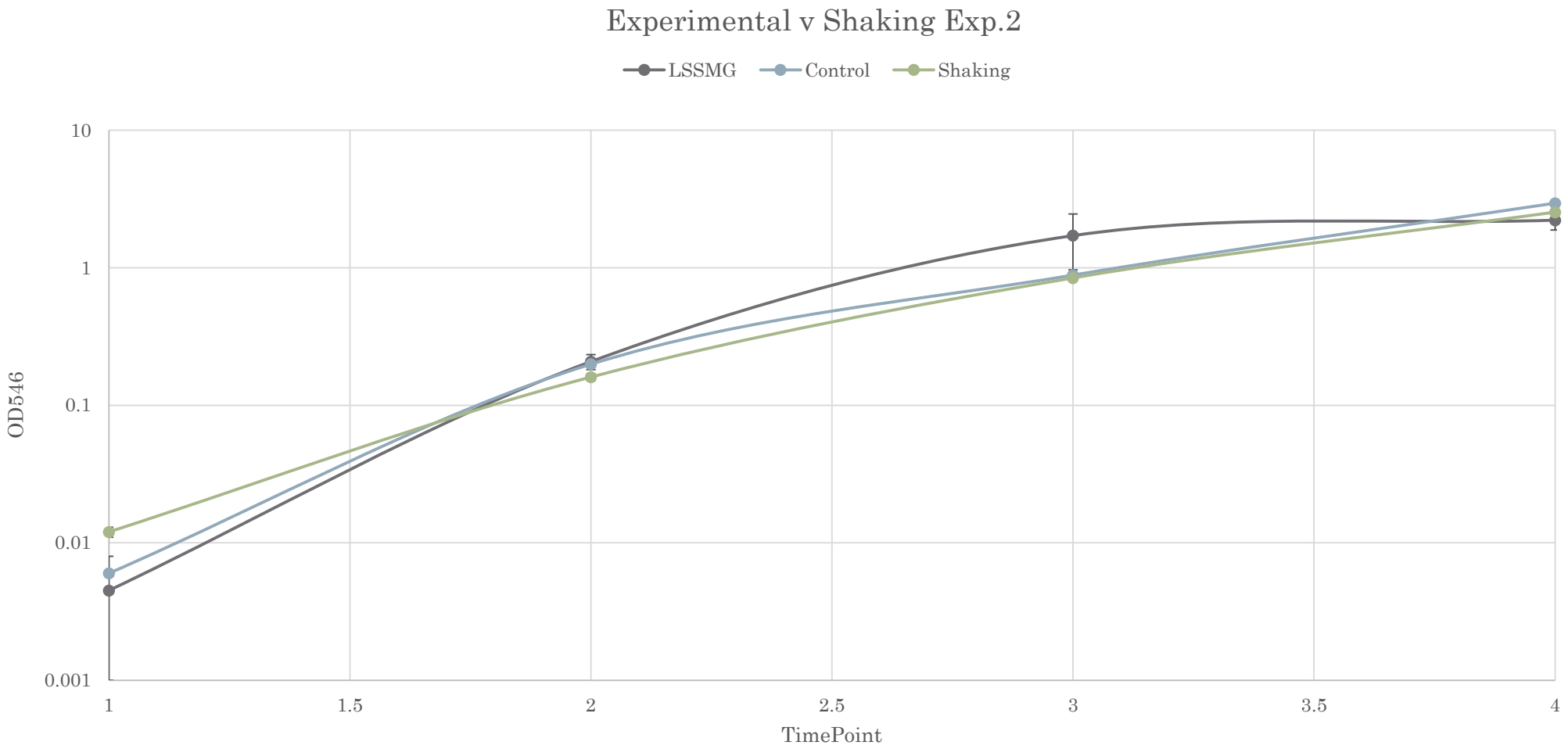
Experimental Design – Exp. 2

- "Time Point" Experiment
  - Chose 4 time points
  - 2 vessels per time point
  - Experimental (vertical) and control (horizontal) – 8 vessels total
  - Removed 2 vessels at each time point
  - Harvested the entire vessel – OD, plated for counts and for antibiotic test
  - Also ran a shaking culture

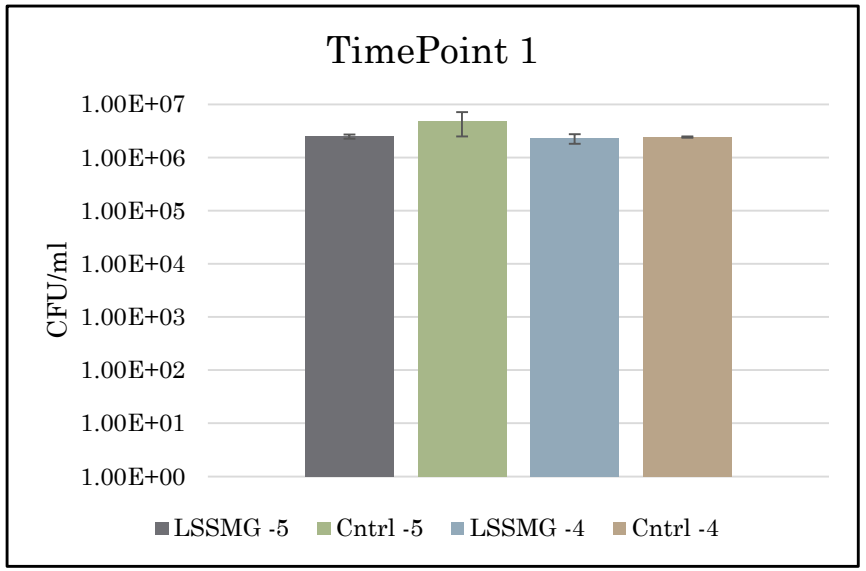


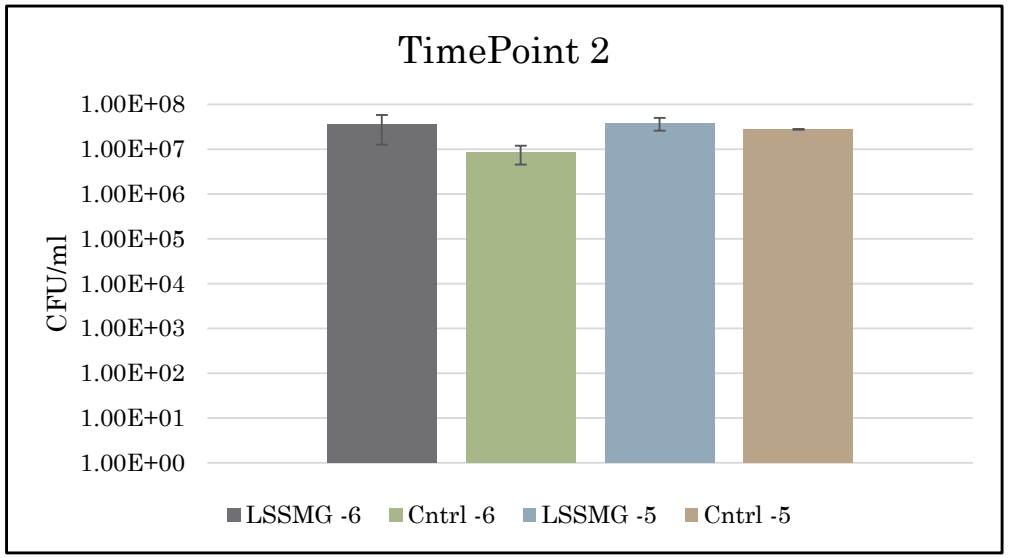


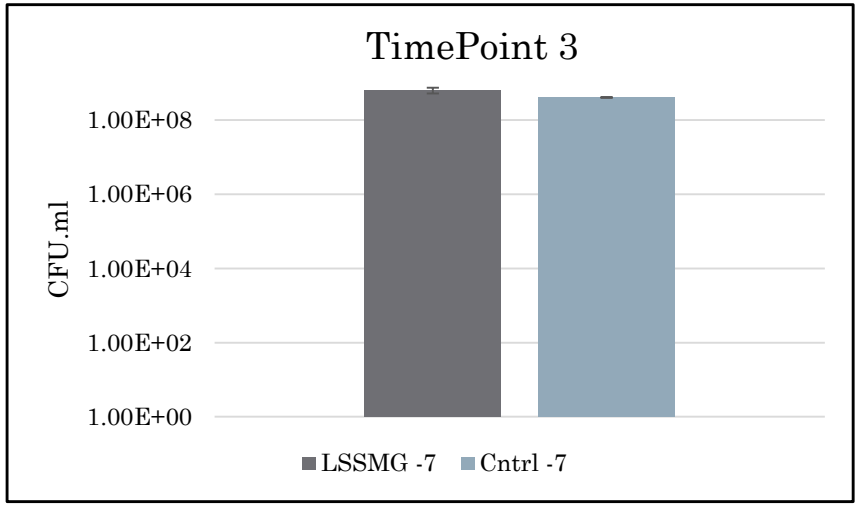
Exp. 2 Results –Growth Curve



Exp. 2 Results – Counts





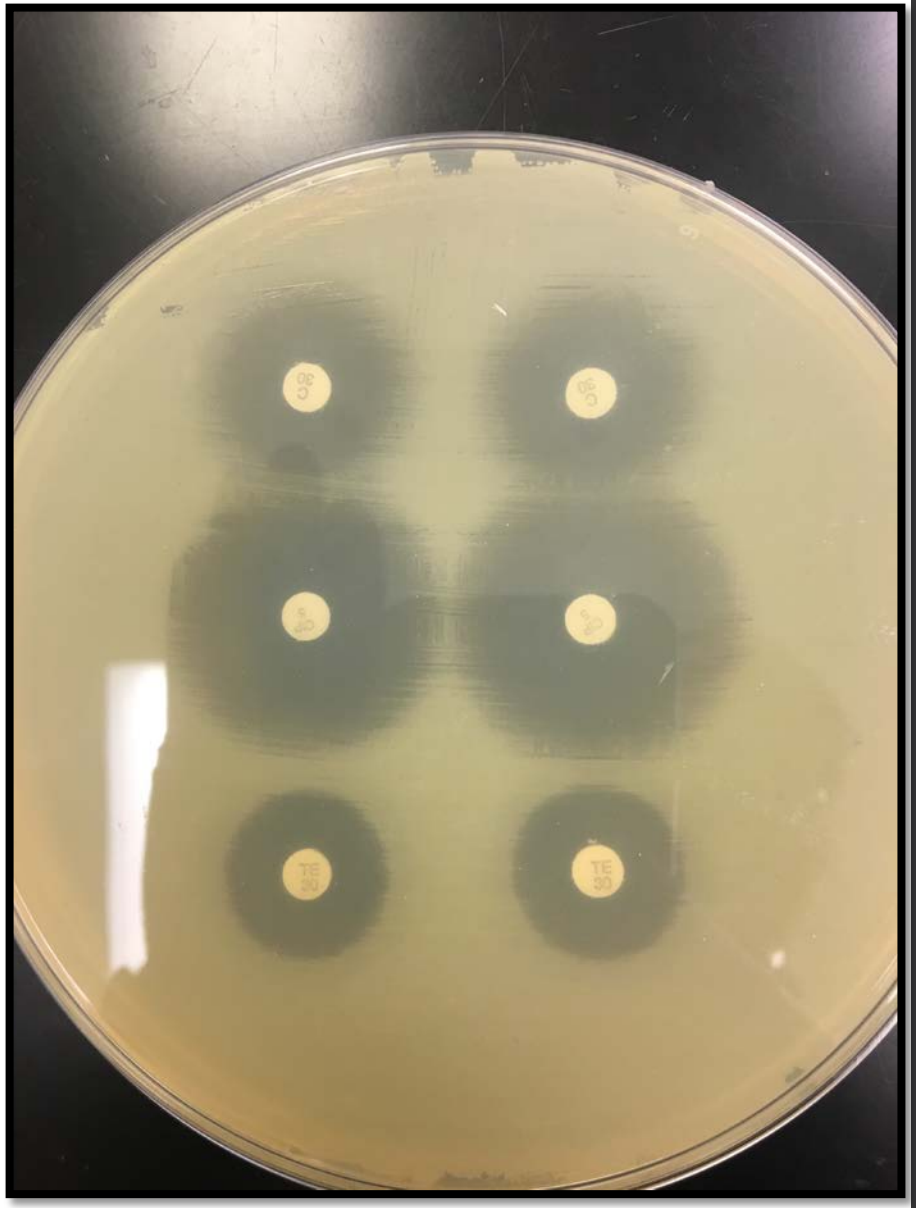


Exp. 2 – Cont.

- Antibiotic Test
  - Kirby-Bauer Antibiotic Susceptibility Test
  - Used to determine antibiotic susceptibility of bacteria
    - Measuring Zone of Inhibition (ZOI)
  - Mueller-Hinton Agar is used.

- Antibiotics
  - Ciprofloxacin (5µg)
  - Tetracycline (30µg)
  - Chloramphenicol (30µg)

- Tested *E. coli* ATC25922 for quality control



Exp. 2 Results – Antibiotics (ZOI)

Antibiotic	Susceptibility	Intermediate	Resistant
Tetracycline (30µg)	≥15mm	12-14mm	≤11mm
Ciprofloxacin (5µg)	≥21mm	16-20mm	≤15mm
Chloramphenicol (30µg)	≥18mm	13-17mm	≤12mm

*

*Based on standards set by the CLSI MS100 for the *Enterobacteriaceae* family.

<u>*P. agglomerans* ZOI Average</u>

- Control
  - Tetracycline: 22.4mm= S
  - Ciprofloxacin: 34.9mm= S
  - Chloramphenicol: 24.3mm= S
- LSSMG
  - Tetracycline: 21.1mm= S
  - Ciprofloxacin: 34.4mm= S
  - Chloramphenicol: 24.4mm= S
- Shaking Ground Control
  - Tetracycline: 22.4mm= S
  - Ciprofloxacin: 35.4mm= S
  - Chloramphenicol: 24.9mm= S

Conclusions

- So far:
  - Growth Curves: H_{1A} looks promising (Hypothesis 1 is accepted)
  - Antibiotic Resistance: H_{0B} looks likely (Null Hypothesis is accepted)
- Some significant differences in the growth rate in LSSMG compared to the control during 1hr intervals and media addition.
- No significant change in growth rate in LSSMG compared to the control when ran continuously, chose time points and no media addition
- No change in antibiotic susceptibility.
- Tale of two experiments.

Future Work

- Repeat, Repeat and Repeat again
- Continuously run bacterial culture in RCCS
  - Antibiotic resistance may not have had time to develop
- Addition of an antibiotic resistant microbial species.
  - Test in LSSMG if there is gene transfer of antibiotic resistance gene to *P. agglomerans*
- Addition of Media vs Harvesting entire Vessel
- Kirby-Bauer on Addition of Media Experiments

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Questions?

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