Predation Enhances Survival and Growth of Pathogenic and Non-Pathogenic Isolates of *Vibrio cholerae*

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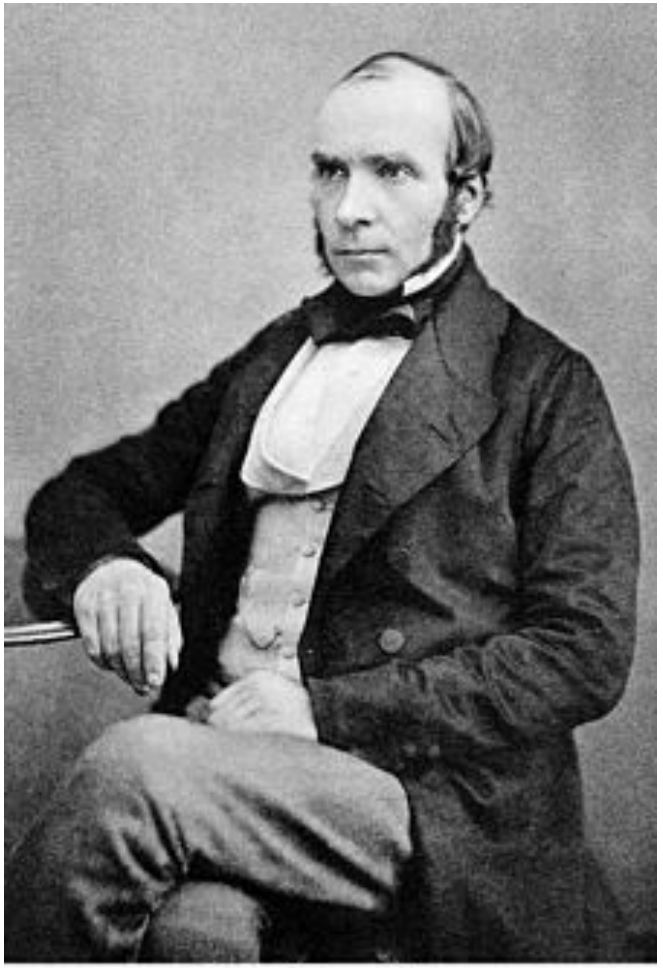
USDA United States Department of Agriculture National Institute of Food and Agriculture

John Snow and Cholera:

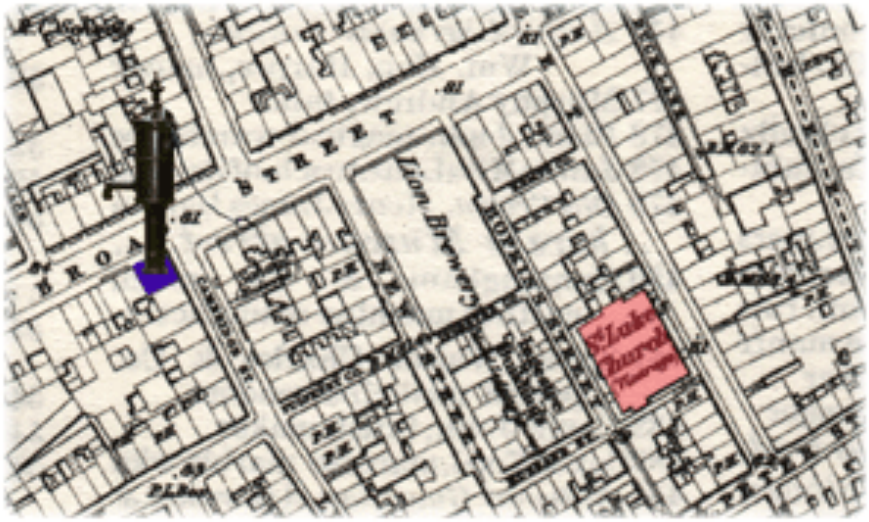
- John Snow postulated Cholera to be a digestive disease (enters through oral-fecal route).
- 1854: Third Pandemic in Southern London.
- Outbreak in Albion Terrace: "The Grand Experiment."
  - Linked deaths to water companies.
  - Postulated Cholera with contaminates in water



Kukaswadia, Atif. "John Snow: The First Epidemiologist." Public Health Perspective. Web. http://blogs.plos.org/publichealth/2013/03/11/john-snow-the-first-epidemiologist/

Broad Street Outbreak:

- Broad Street Outbreak.
  - Made a map with black marks representing a death.
  - At first deaths seemed unrelated to pump.
  - Linked deaths to Broad Street Pump.
  - Supported his postulate linking Cholera to drinking water.



UCLA. Web. http://www.ph.ucla.edu/epi/snow/whitehead.html

Vibrio cholerae Infections:

: Area's with known Cholera cases.

"Cholera – Vibrio cholerae infection." CDC. Web. http://www.cdc.gov/cholera/index.html

Vibrio cholerae Cycle:

The Phytoplankton Encyclopedia Project." University of British Columbia. Web
https://www.eoas.ubc.ca/research/phytoplankton/at_a_glance/zooplankton_detritus.html.

1. Plankton associated biofilms

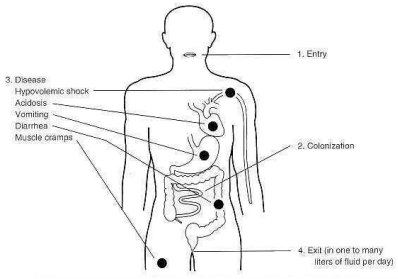
2. Environmental changes affecting *V. cholerae* populations

"Pakistan Flood Devestation." BBC News. Web.
http://www.bbc.co.uk/news/world-south-asia-11052984

3. Renewed cholera outbreaks

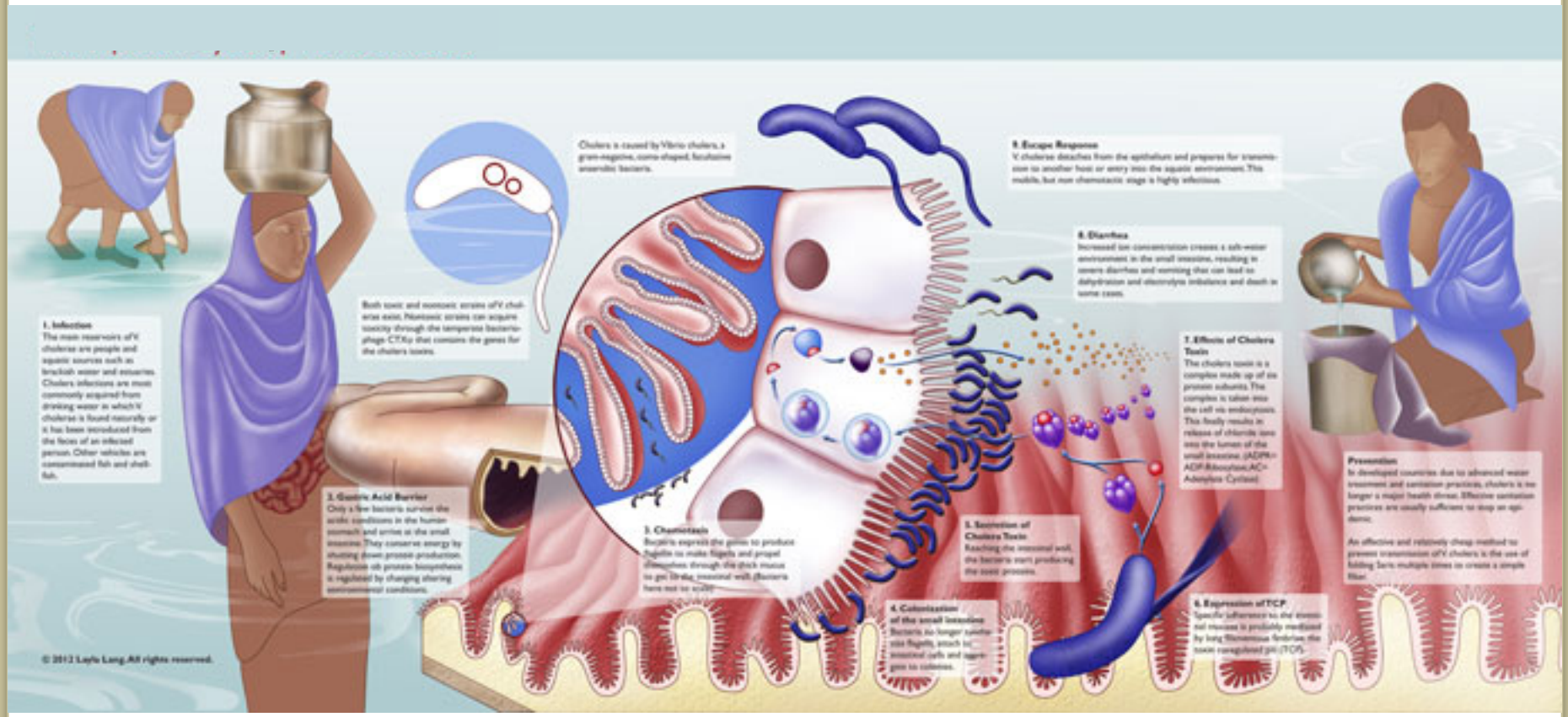
Yakubu Busari, Jos. "Cholera Outbreak Hits Jos, Several Persons Admitted to Hospitol." Universal Reporters. Web.
http://universalreporters247.com/2015/06/cholera-outbreak-hits-jos-several-persons-admitted-in-hospital/

4. Pathogenicity and cholera



"Cholera." Malonie Health. Web.
http://www.malonie.com/health/Cholera/

Infection Cycle of *Vibrio cholerae:*

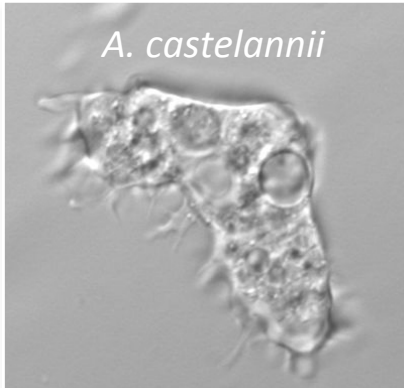


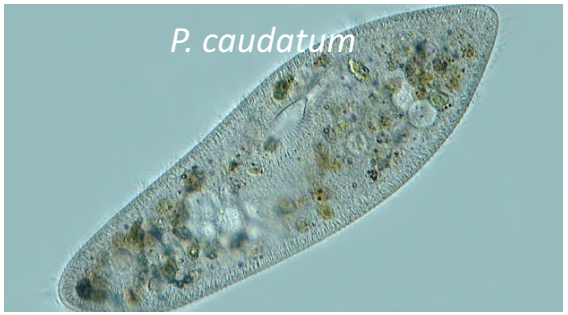
Lang, Layla. "The Infection Cycle of Vibrio cholerae." Science Art. Web. http://www.science-art.com/image/?id=5302&pagename=The_Infection_Cycle_of_Vibrio_cholerae&m=24866#.Vz23-ecrL-l

How do environmental pressures in general affect bacterial growth and survivability?

Top Down Interaction Benefit Bacteria:

- Protozoa grazing (Predator).
- Bacteria benefit from such interaction:
  - Ability to escape predation.
  - Growth under predator.
  - Ability to resist intracellular digestion (intracellular survival in trophozoite or cyst formation).



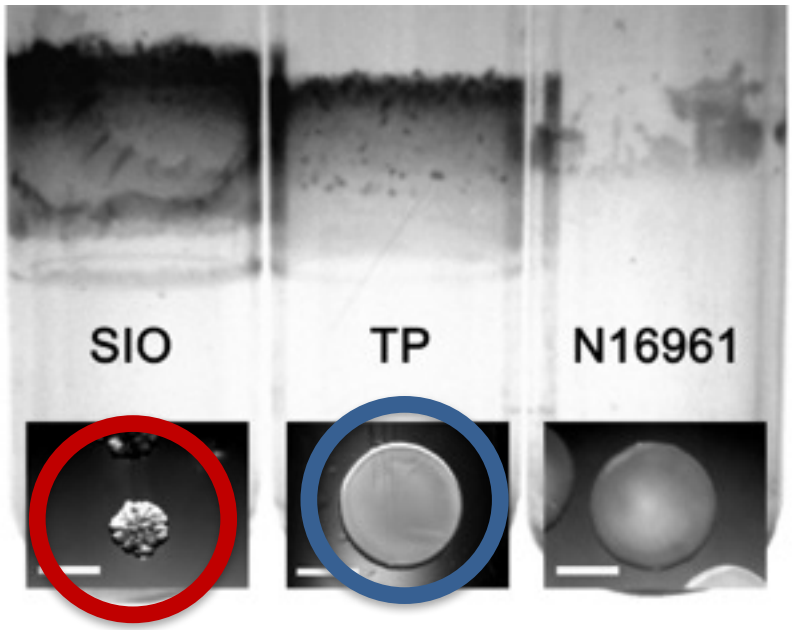


"P. caudatum." Pling Factory. Web. http://www.plingfactory.de/Science/Atlas/KennkartenProtista/01e-protista/e-Ciliata/e-source/Paramecium%20caudatum.html

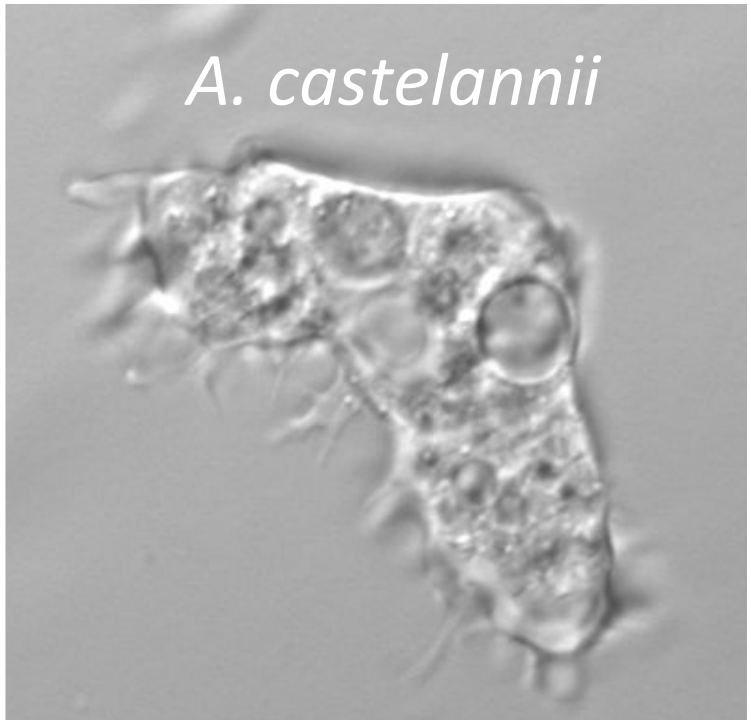
Bacterial Phenotypes:

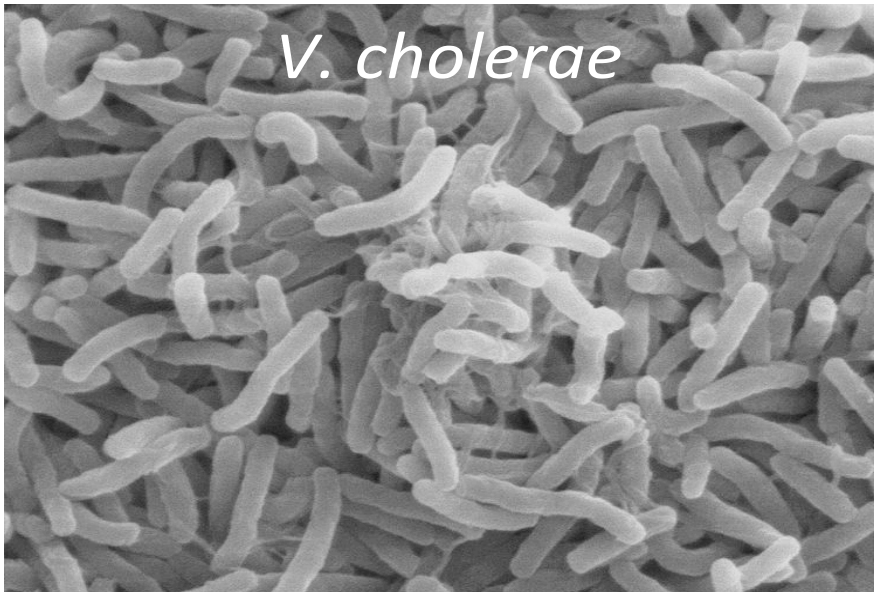
- Biofilm formation (Rugose or Smooth).
- Biofilm acts as environmental refuge (promotes persistence).
- Environmental factors select for biofilm phenotypes.

V. cholerae strs.



Predator Prey Selection:





Objectives:

I. Are *V. cholerae* strain variants equally sensitive to grazing pressure by *A. castellanii.*
II. Does ingestion of these bacteria affect amoebae cells causing a change in growth, or its physiological state.
III. Does biofilm-formation ability confer against amoebae grazing in *V. cholerae* strain variants.

Sub-objectives:

- Analyze *V. cholerae* characteristics.
- Examine if amoebae are active and feeding on *V. cholerae.*

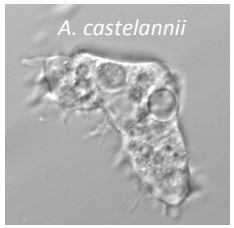
Strain Designation:

Table 1. Bacteria, amoebae, and plasmids used in this study.

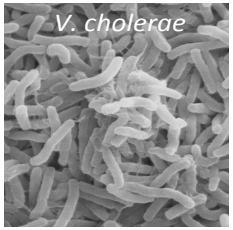
Strain Designation or Plasmid	Relevant Genotype or Description	Reference
A. castellanii		
Neff	Wild-type	[20]
E. coli		
S17-1λπ	*recA pro hsdR* RP4-2-Tc::Mu-Km::Tn7, p519n*gfp* plasmid	[23]
V. cholerae		
S4100	SIO strain, *gfp* inserted downstream of ribosomal rRNA promoter, non-pathogenic to humans	[19]
S100f1	SIO strain, p519n*gfp* plasmid, non-pathogenic to humans	[19]
T100f1	TP strain, p519n*gfp* plasmid, non-pathogenic to humans	[19]
AS4100	O1 El Tor, smooth variant, *gfp* downstream of ribosomal rRNA promoter, pathogenic to humans	[27]
AR4100	O1 El Tor, rugose variant, *gfp* downstream of ribosomal rRNA promoter, pathogenic to humans	[27]
Plasmids		
p519n*gfp*	RSF1010 derivative containing *pnptII* promoter before *gfp* gene, *mob*, Km^r	[16]

Methods:

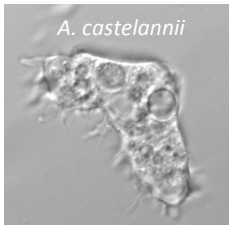
Predator

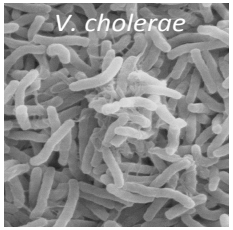


Prey



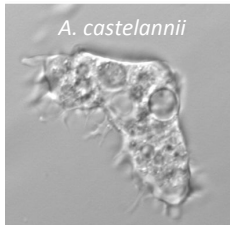
Co-cultured





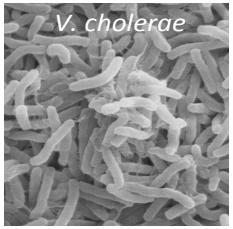
Methods Continued:

Predator



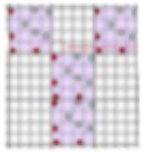
10^5 cells/mL

Prey



10^4 cells/mL

Amoeba Counts

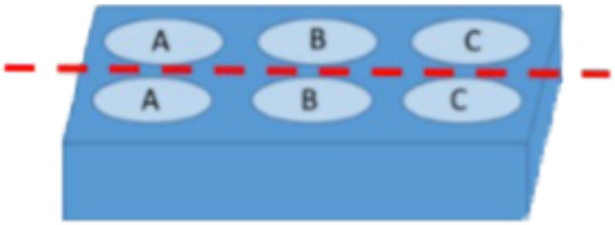


t = 0 hours

Replicate

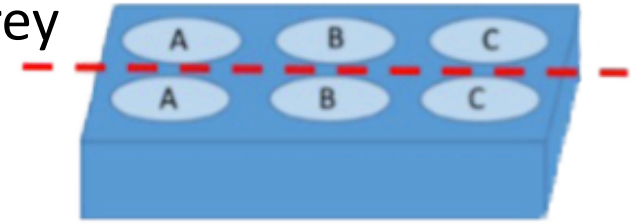
Predator

No Predator



Predator & Prey

Prey

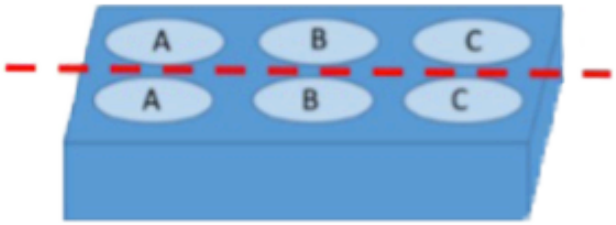


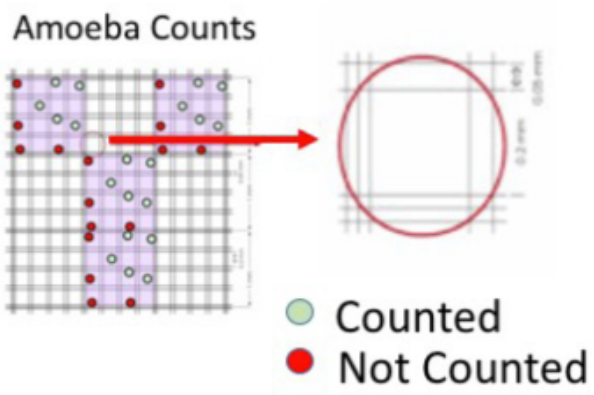
Methods Continued:

t = 24, and 48 hours

Predator

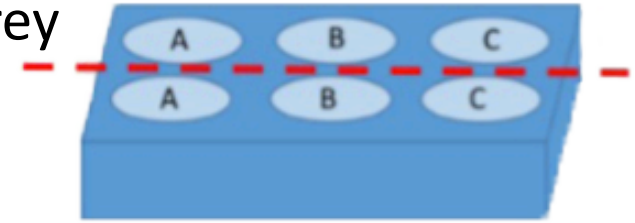
No Predator





Predator & Prey

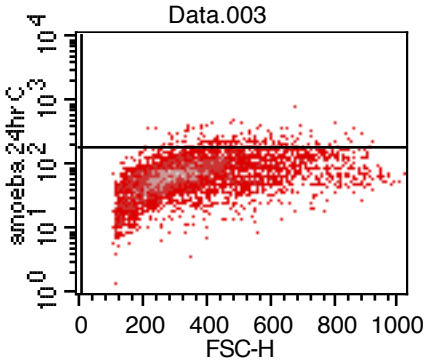
Prey



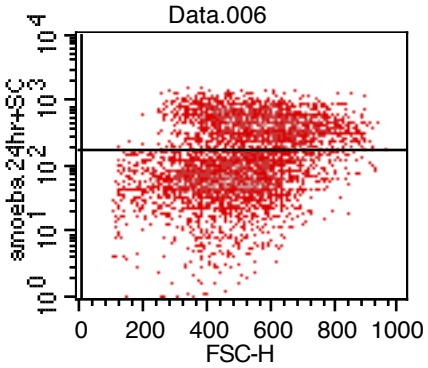
Results:

A. castellanii Actively Internalize *V. cholerae* Strain SIO Cells

24 hour

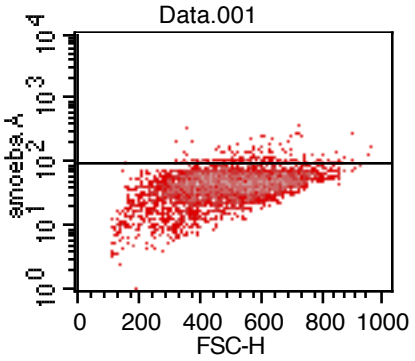


Amoebae alone

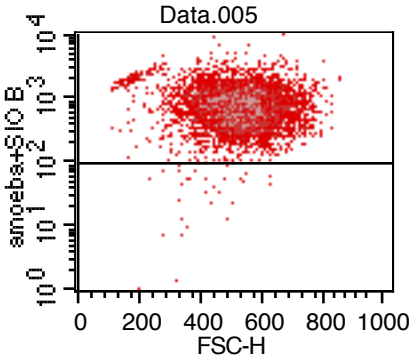


Co-culture

48 hour

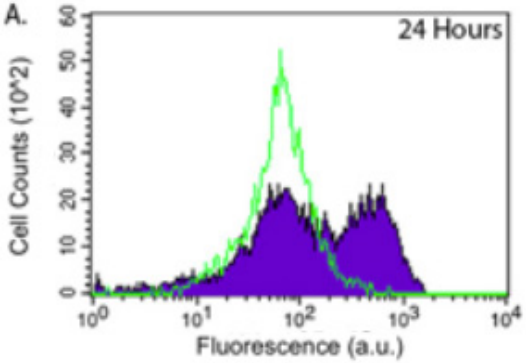


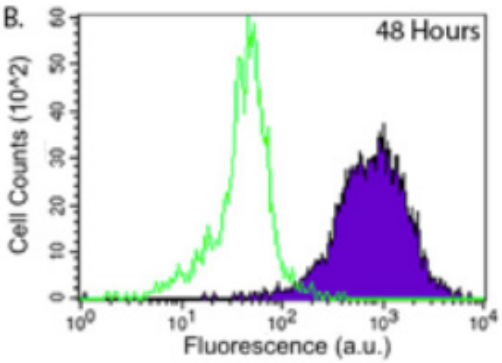
Amoebae alone



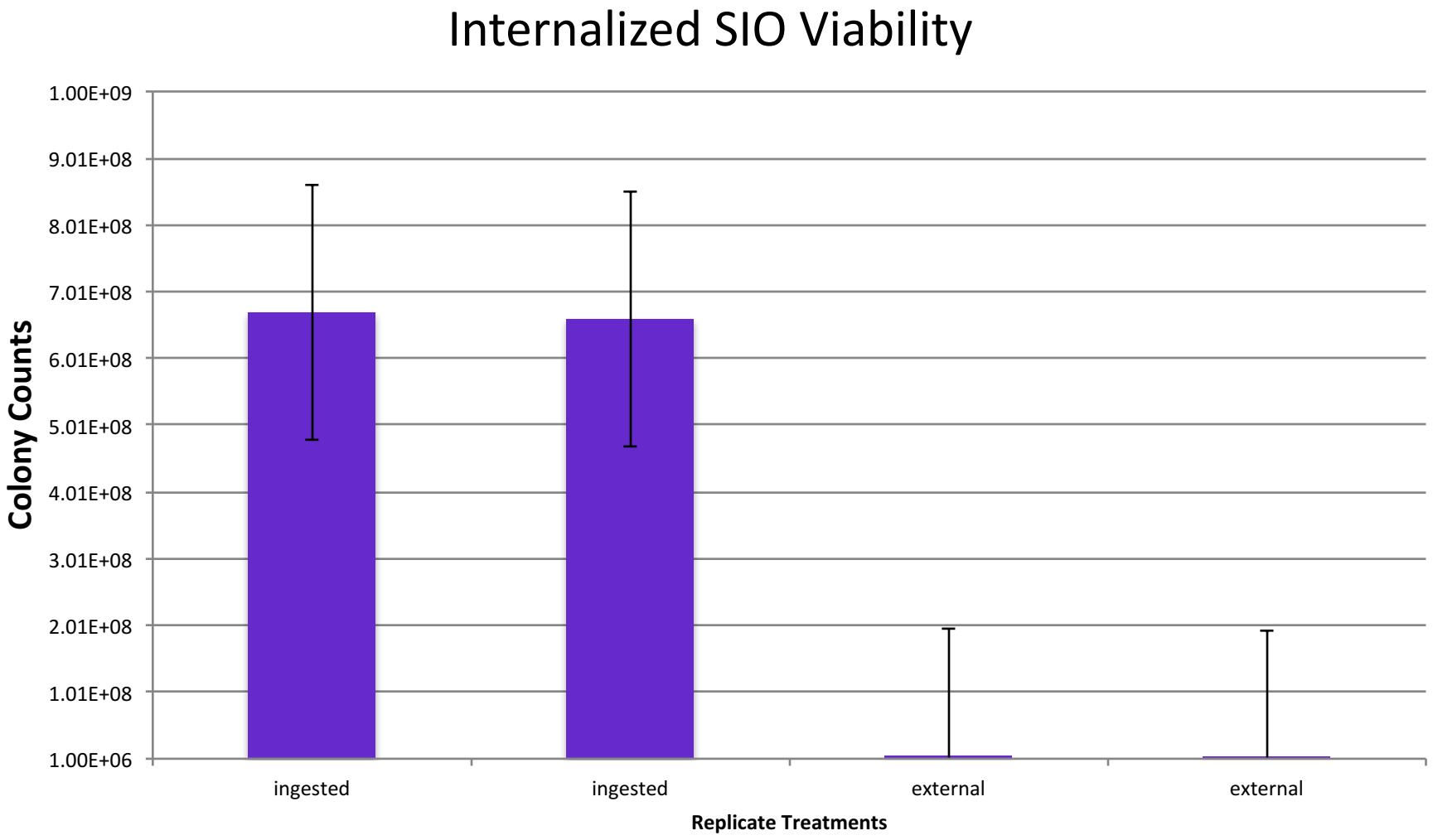
Co-culture

Quantification of Fluorescent *A. castellanii*

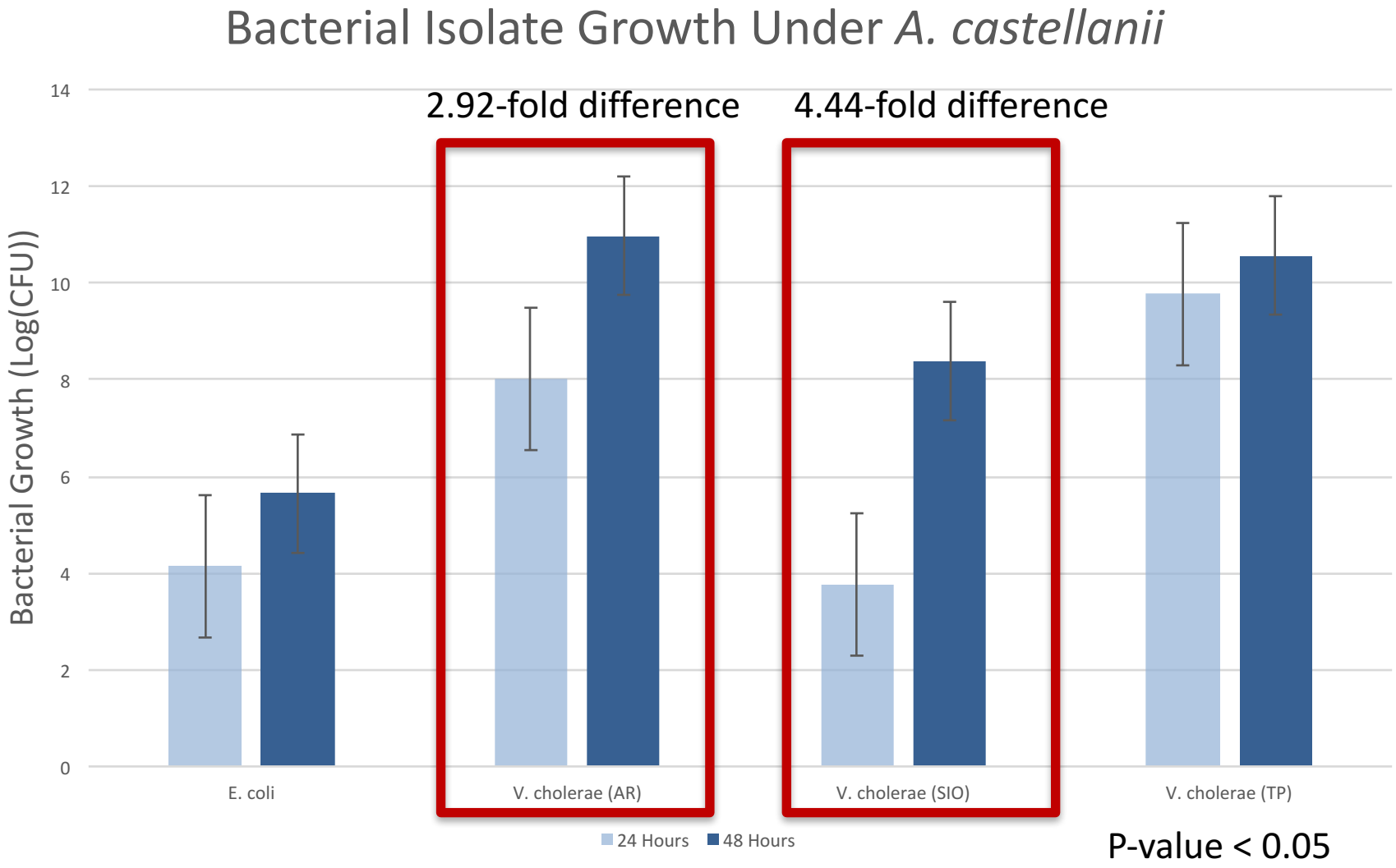




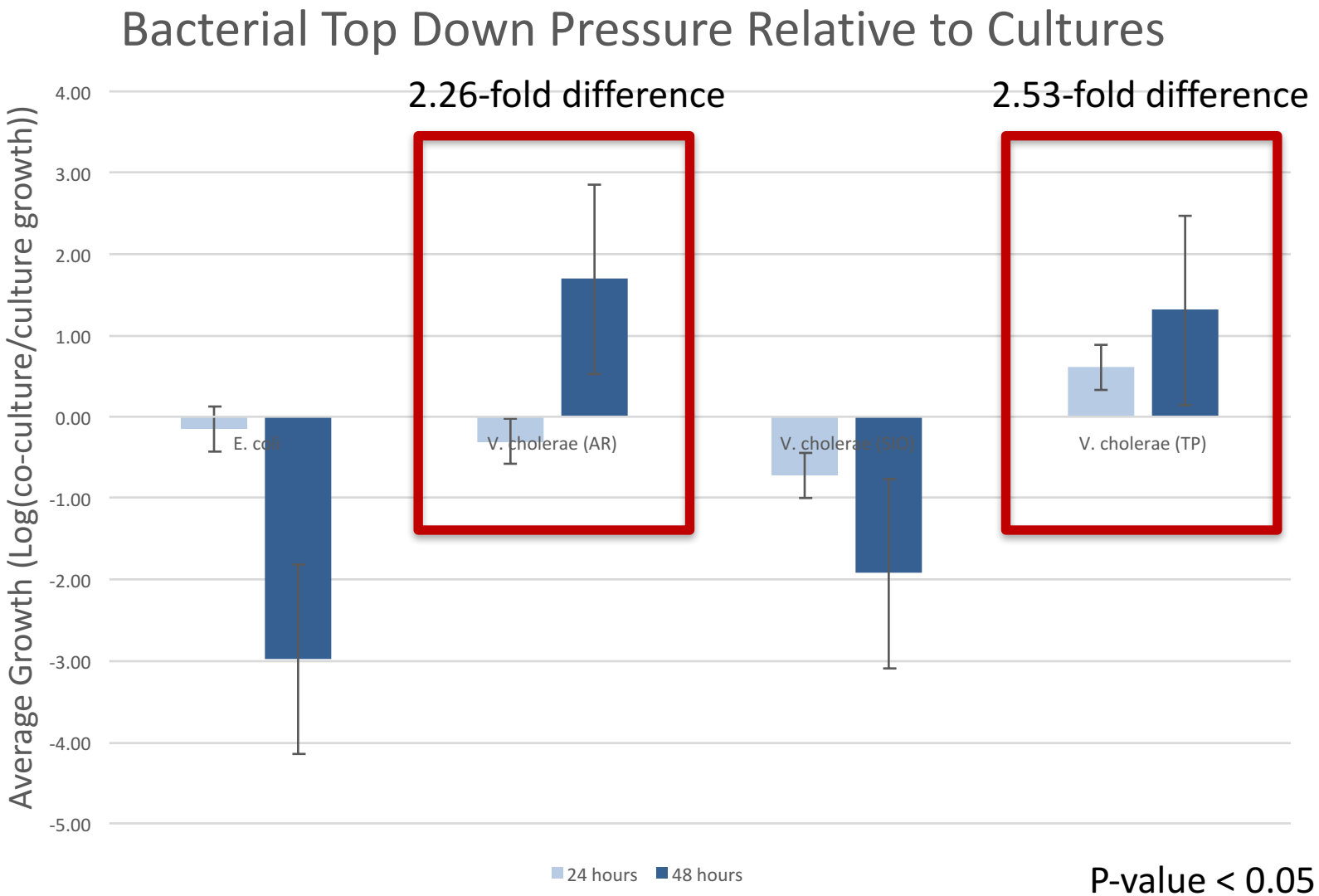
Results:


Internalized SIO Viability
1.00E+09
9.01E+08
8.01E+08
7.01E+08
6.01E+08
5.01E+08
4.01E+08
3.01E+08
2.01E+08
1.01E+08
1.00E+06
Colony Counts
ingested
ingested
external
external
Replicate Treatments

Results:

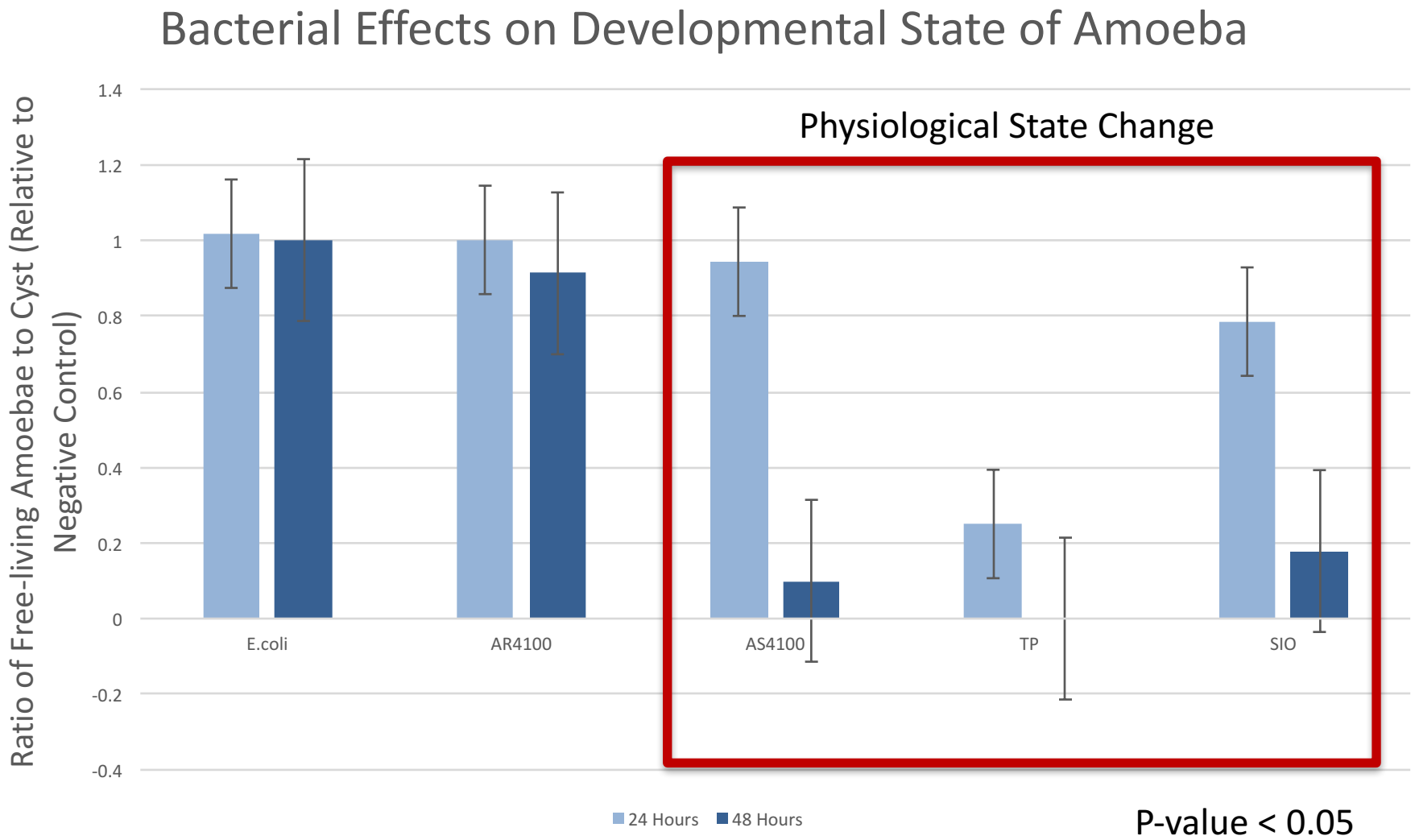

Bacterial Isolate Growth Under A. castellanii
2.92-fold difference
4.44-fold difference
Bacterial Growth (Log(CFU))
14
12
10
8
6
4
2
0
E. coli
V. cholerae (AR)
V. cholerae (SIO)
V. cholerae (TP)
24 Hours
48 Hours
P-value < 0.05

Results:



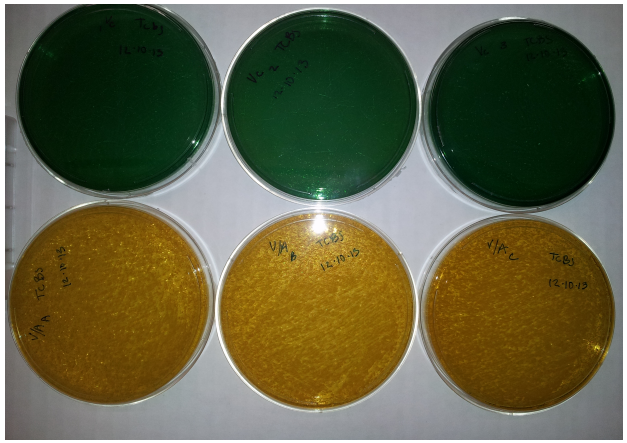
P-value < 0.05

Results:


Bacterial Effects on Developmental State of Amoeba
Ratio of Free-living Amoebae to Cyst (Relative to Negative Control)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
Physiological State Change
E.coli
AR4100
AS4100
TP
SIO
24 Hours
48 Hours
P-value < 0.05

Co-Culture Conditions Induce Significant Physiological Differences in *V. cholerae*

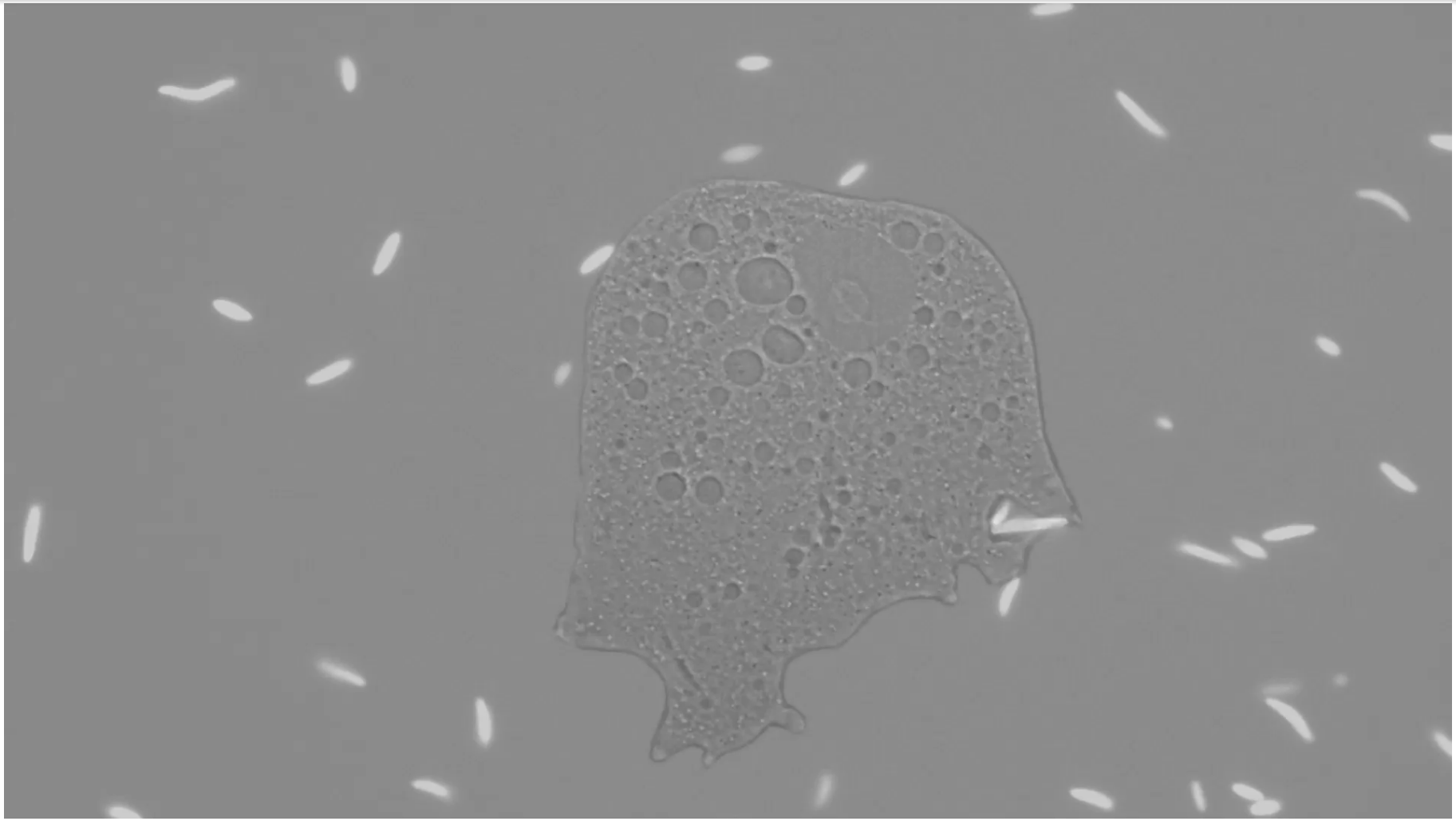
- After 6 days of co-culture cells with amoebae retain the ability to be cultured
- Cells in co-culture conditions were large, fluorescent rods
- Cells in negative control cultures (bacteria alone) were small cocci that had lost fluorescence



Vc alone

Vc/Ac co-culture

Observed *A. castellanii* feeding behavior on *V. cholerae:*



Conclusion:

- Grazing sensitivity is strain specific
  - Certain *Vibrio cholerae* strains proliferate under grazers (selection)
  - Cause cyst formation in Amoeba (intracellular protection).
- Mechanism of resistance (not biofilm alone)
- Long term survival of vibrios (physiological changes)
  - Survive harsh environments, and are still viable (produces selective strain).
  - Amoeba acts a pathogenic vector for *Vibrio cholerae*.

Acknowledgement:

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- Bioenergy Minor Program
- Minorities in Agriculture, Natural Resources and Related Sciences (MANRRS)
- Oregon State University

Questions?

Further Information:

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