Exploring the links between growth rate, metabolic phase and resistance of *Pseudomonads*

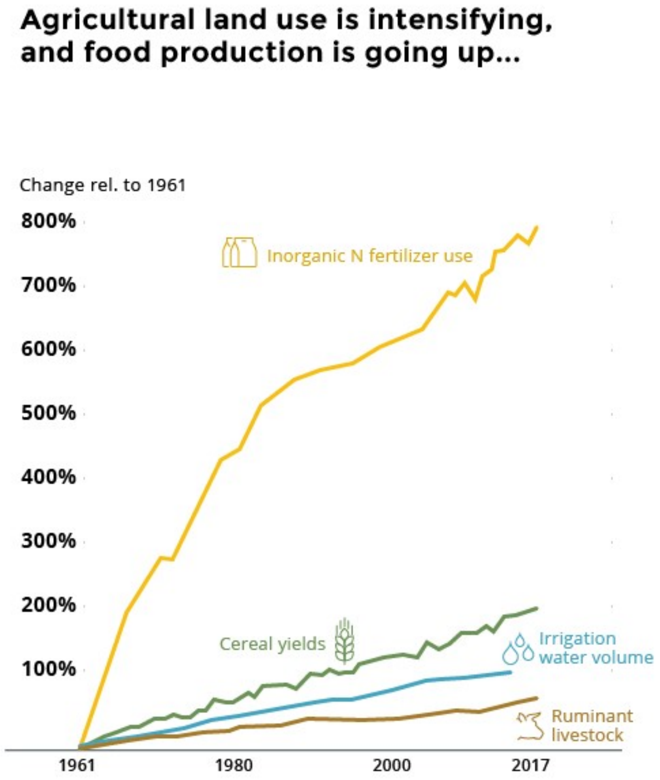
Anjaney Pandey

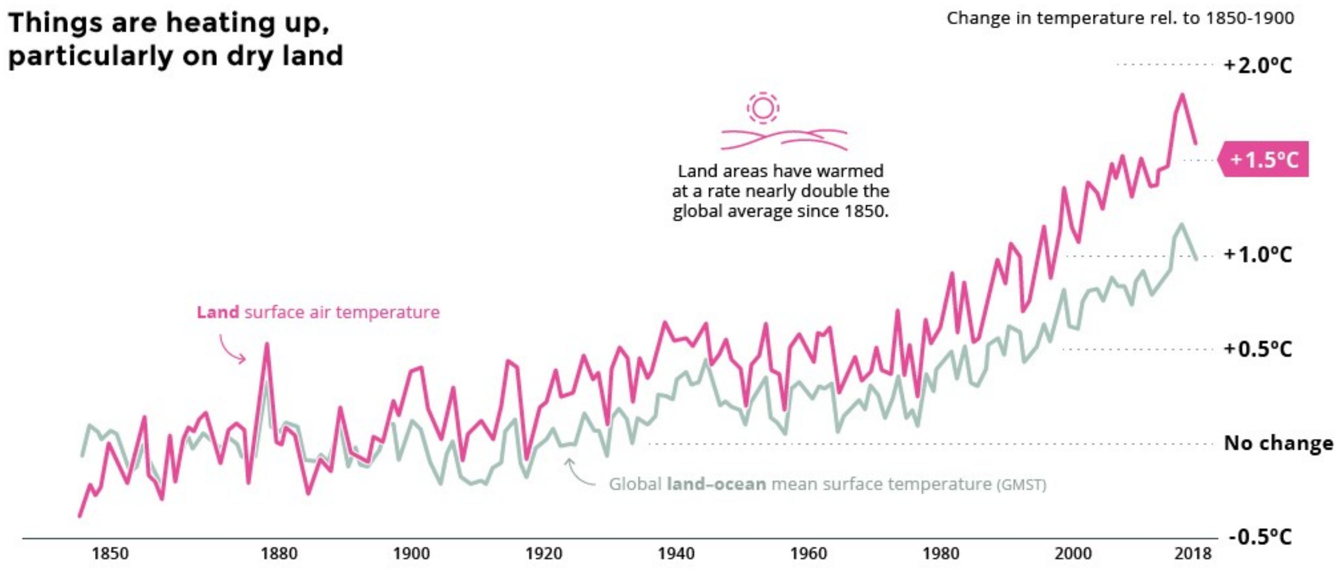
6th December 2023

OVERVIEW

- Introduction
- Growth curves
- Hypothesis
- Main Experimental Design
- Methods
- Real growth curves - Snapshot
- TTD analysis
- Resistance
- Plots
- Future direction
- Discussion
- Acknowledgement

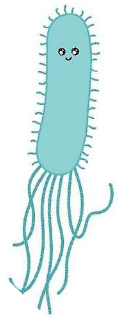
What’s happening around?





https://www.visualcapitalist.com/impact-on-climate-change-and-land-use/

Why *Pseudomonads*?



Pseudomonas is a genus of bacteria that are widely found in soil and water.

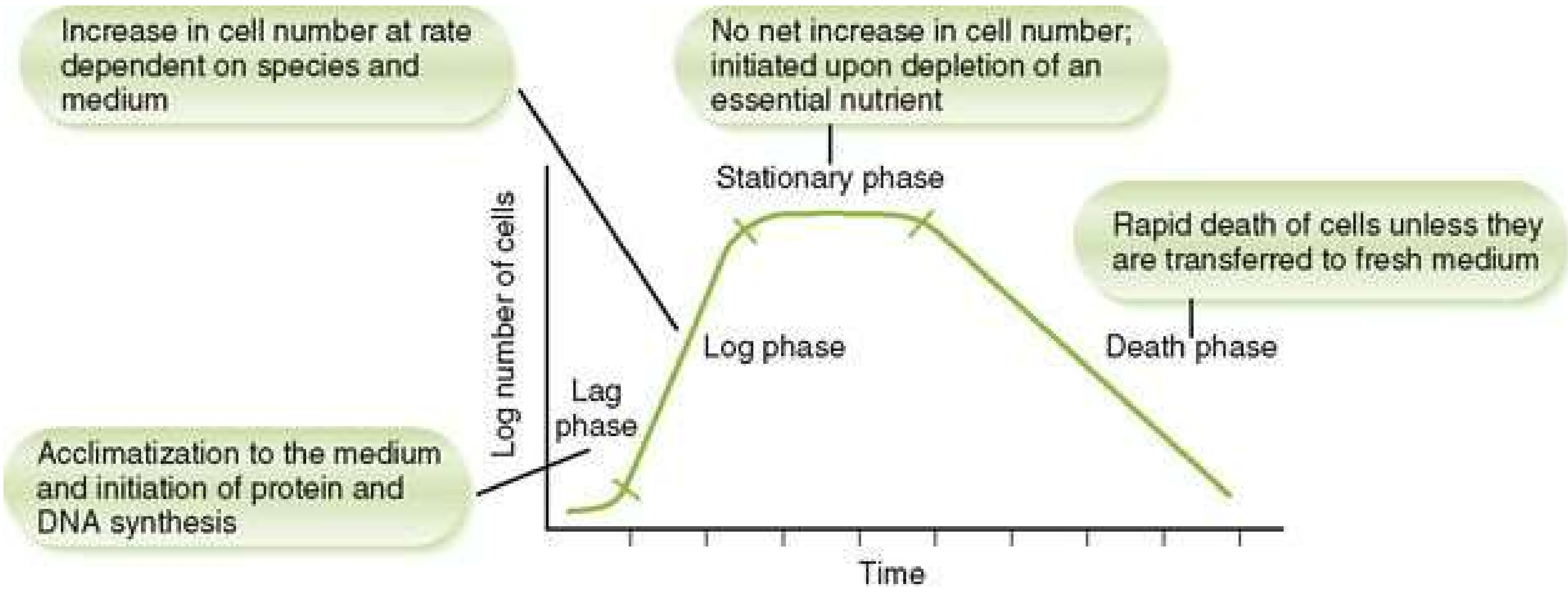
- **Metabolically versatile:** can degrade various organic compounds, including hydrocarbons and pesticides. This allows them to adapt to different soil conditions and environmental stresses.
- **Plant-associated:** Produce phytohormones, siderophores, antibiotics, and other secondary metabolites that can enhance plant nutrition, protection, and resistance.
- **Cosmopolitan**: represent a large and varied group of soil bacteria that can reflect the changes in soil microbial community structure and function under heat stress.
- **Easily detectable** by their fluorescent pigments and molecular markers.

Narrative of a versatile and adept species Pseudomonas putida.
Maia Kivisaar, 2020

The versatility of *Pseudomonas putida* in the rhizosphere environment.
Molina et.al., 2020

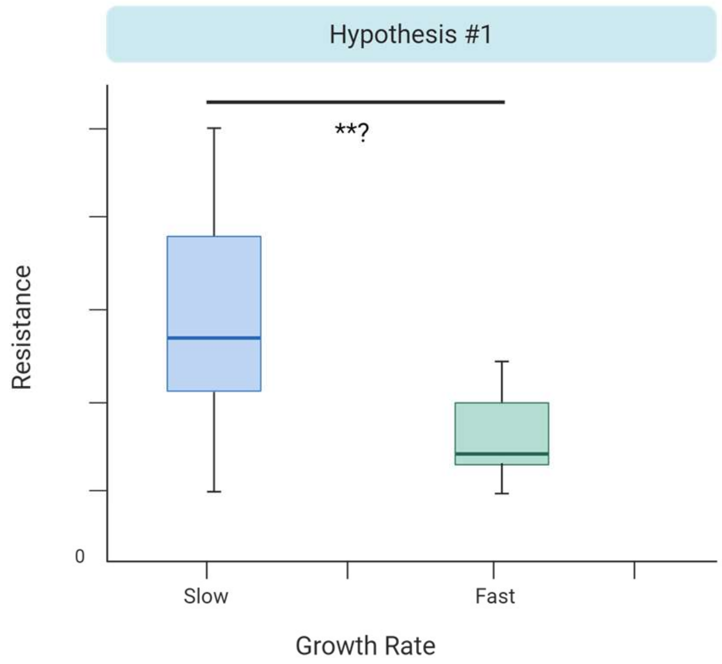
***Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security.**
Sah et.al., 2021

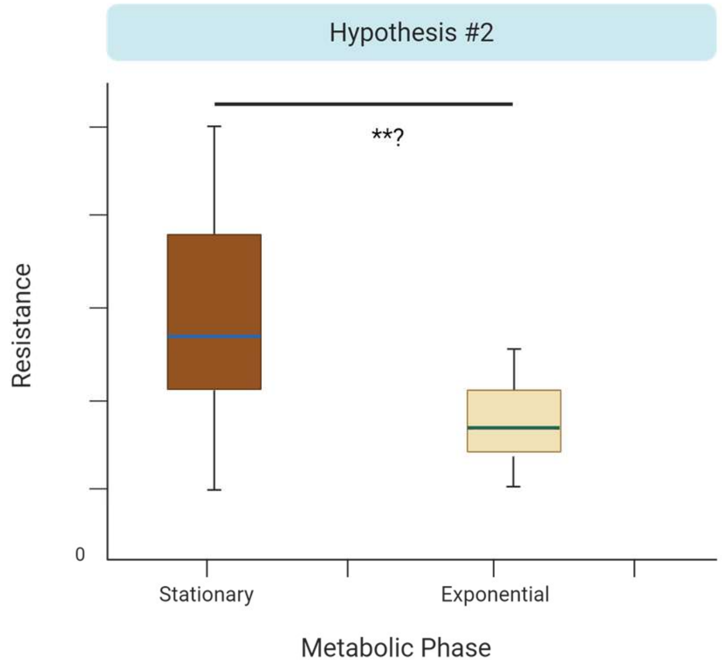
Growth Curves



- fast and slow – based on μ_max
- different metabolic phases – based on how close to K

Hypothesis


Hypothesis #1
**?
Resistance
0
Slow
Fast
Growth Rate


Hypothesis #2
**?
Resistance
0
Stationary
Exponential
Metabolic Phase

Rationale

- In the stationary phase, the cell wall is highly cross-linked, membrane fluidity reduces, and the peptidoglycan layer increases in thickness. (Van Heijenoort, 1985).

- Alternative sigma factors become active at stationary phase. For e.g., in E. Coli, the transcription factor σ^S.
  - Induction of σ^S is observed under conditions of low pH, heat or cold shock, UV-induced DNA damage, nutrient starvation, high cell density, high osmolarity, etc. (Hengge, 2011).
  - The σ^S-dependent genes have been attributed to morphological changes, iron uptake, carbohydrate metabolism, amino acid transport, and so on, at the onset of stationary phase (Lacour and Landini, 2004).

- Mortality burden: the ratio of mortality to growth rate. As T increases, μ also increases. Hence, slow growers are less affected. (Gore, 2023)

Rationale - Acclimatization

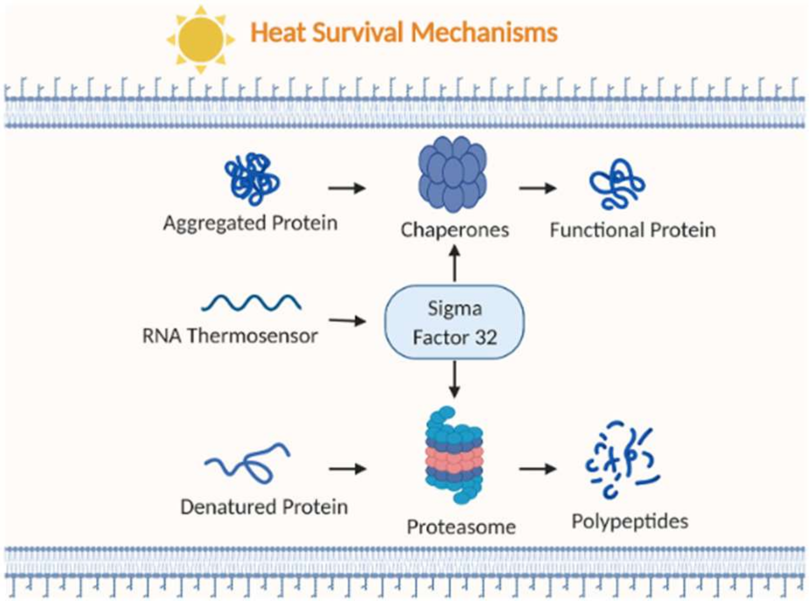


FIGURE 3 | Examples of bacterial strategies to survive heat stress. Chaperones alter aggregated or misfolded proteins into functional proteins. RNA thermosensors regulate the heat shock sigma factor (σ32). Proteasomes break down denatured proteins into polypeptides. Figure created with BioRender.com.

Exopolysaccharide secretion (Alginate)	Water retention and controls biofilm architecture.	Polymer encapulation for formulation.	Chang et al., 2007; Gulez et al., 2014
Chaperone (GroEL/GroES or DnaK/DnaJ/GrpE systems)	Correct misfolded proteins.	Regulation of cyclic lipopeptides.	Mayer et al., 2000; Alix, 2006; Georgescauld et al., 2014
Protease Systems (ClpAP)	Catalyze the breakdown of proteins.	Regulation of cyclic lipopeptides.	Richter et al., 2010; Meyer and Baker, 2011
Thermosensor (ROSE)	Regulates expression of stress response.	Regulation of rhamnolipid production.	Nocker et al., 2001; Vinella et al., 2005; Narberhaus, 2010

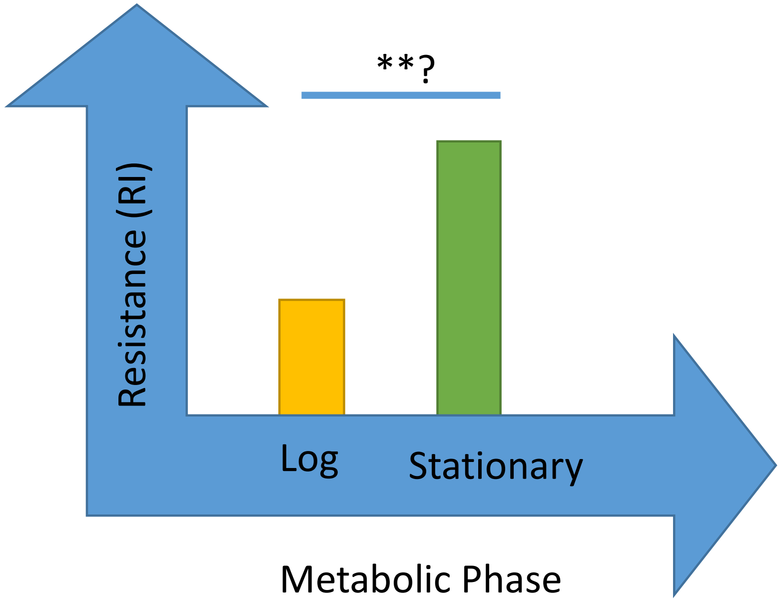
Leveraging *Pseudomonas* Stress Response Mechanisms for Industrial Applications, Craig et. al., 2021
https://doi.org/10.3389/fmicb.2021.660134

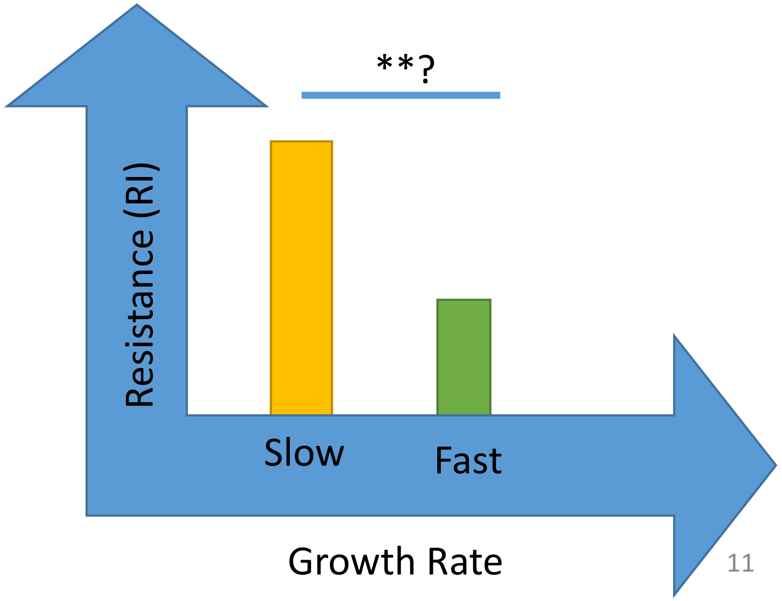
Experimental Design

12 strains in each plate running for 48 hrs.
*1 plate * 4 temperatures * 2 phases * 3 layouts/replicates = 24 plates*

2	3	1	5	8	9	6	10	4	7	15	19

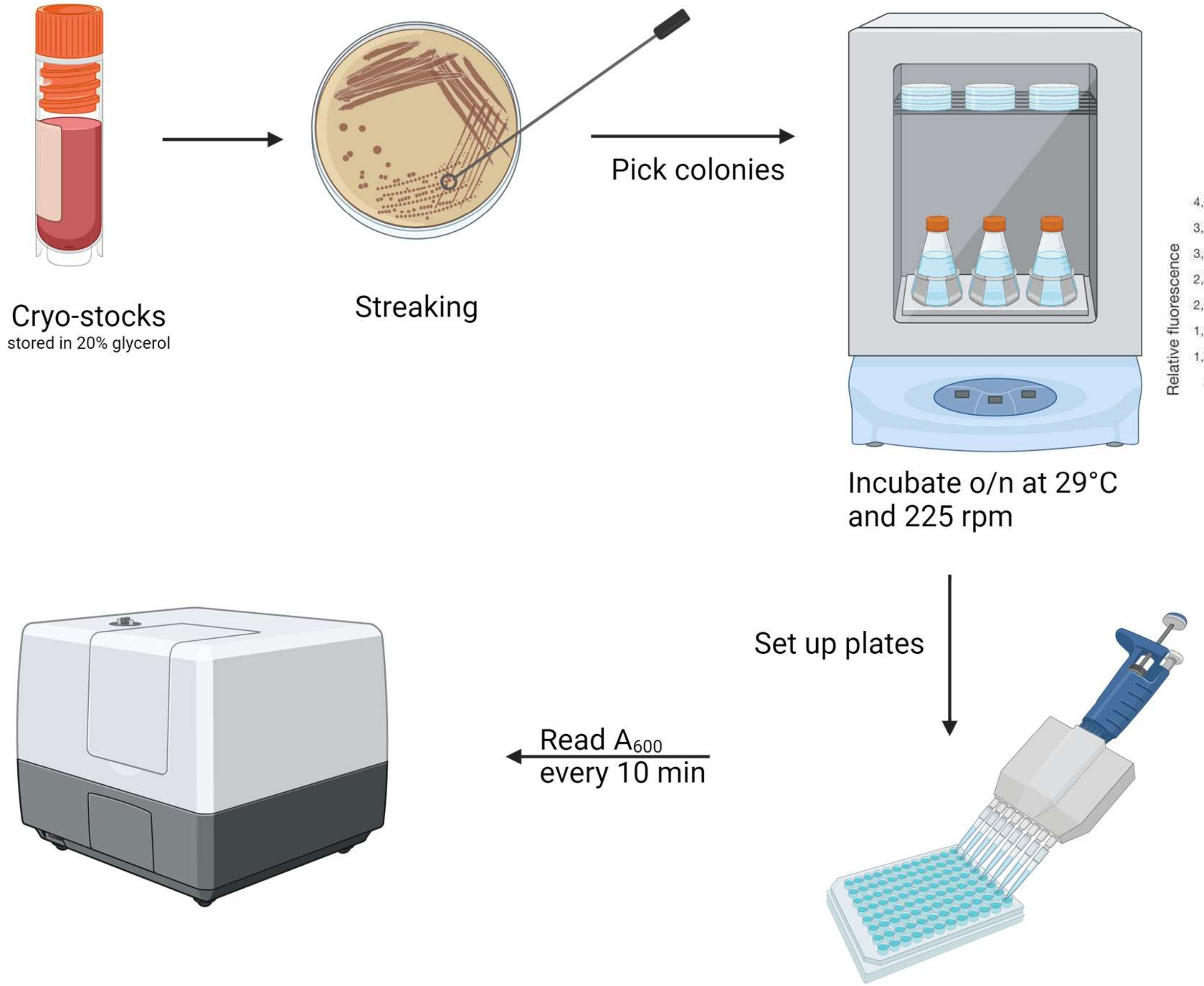
	EXPONENTIAL				STATIONARY			
x3	**25C**	**30C**	**35C**	**40C**	**25C**	**30C**	**35C**	**40C**

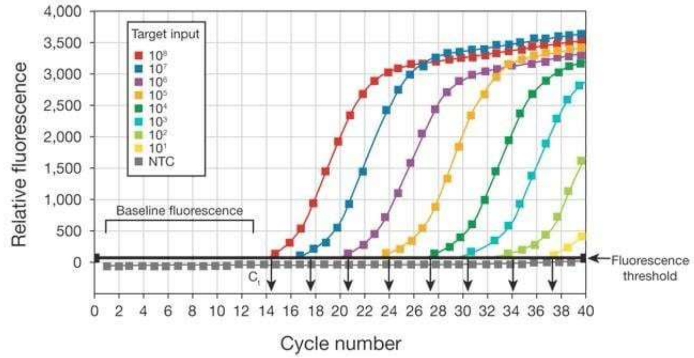




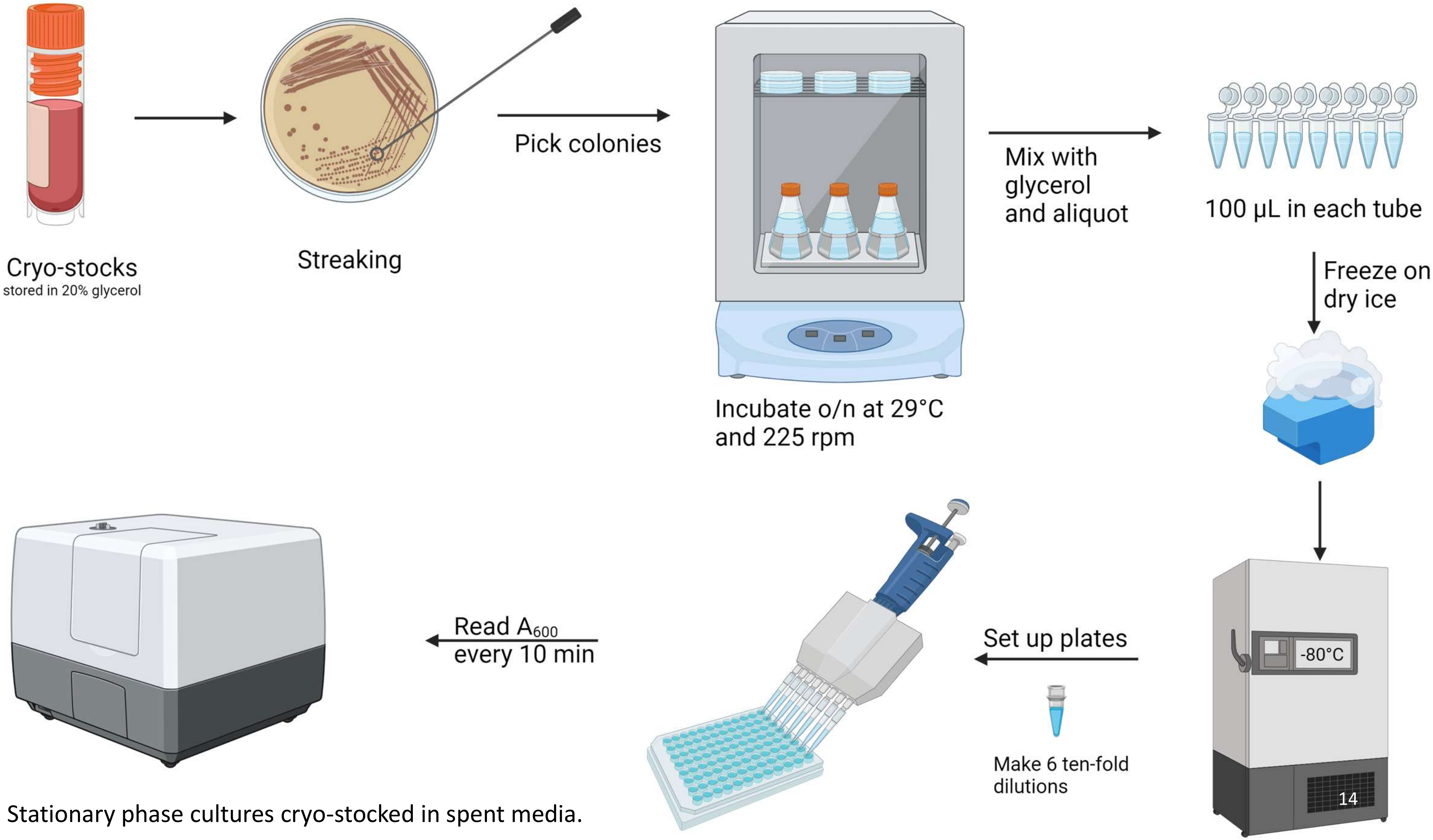
Strains used

ID	Strain identification	Constitutive Fluorescence
BSC001	*Pseudomonas putida F1*	sYFP
BSC002	*Pseudomonas putida KT2440*	mScarlet
BSC003	*Pseudomonas putida uwc 2*	mTagBFP2
BSC004	*Pseudomonas veronii*	mTagBFP2
BSC005	*Pseudomonas veronii*	mScarlet
BSC006	*Pseudomonas veronii*	mCherry
BSC007	*Pseudomonas knackmussii B13*	mCherry
BSC008	*Pseudomonas knackmussii B14*	eGFP
BSC009	*Pseudomonas plecoglossicida*	none
BSC010	*Pseudomonas putida mt-2 KT2440*	none
BSC015	*Pseudomonas putida KT2440*	GFP
BSC019	*Pseudomonas grimontii*	none





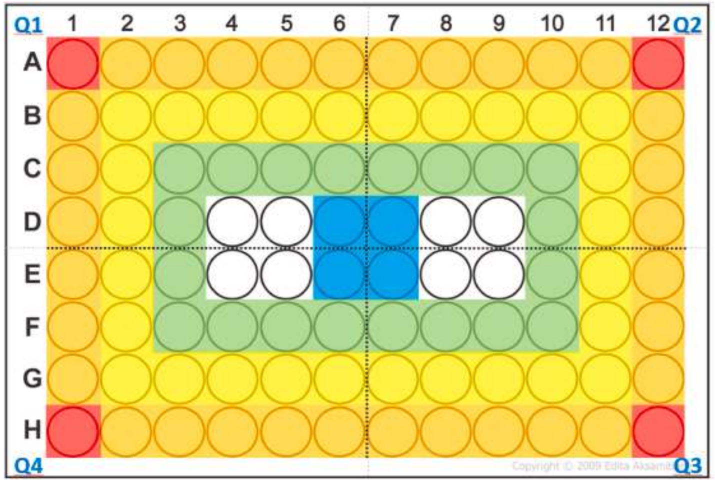
Different dilutions to get around the unidentifiability problem – fitting growth curve to logistic model even if it is not.



Stationary phase cultures cryo-stocked in spent media.

LAYOUTS – to minimize the edge effects on a particular strain.

- Corner and edge wells have been observed to be more prone to evaporation compared to interior wells.

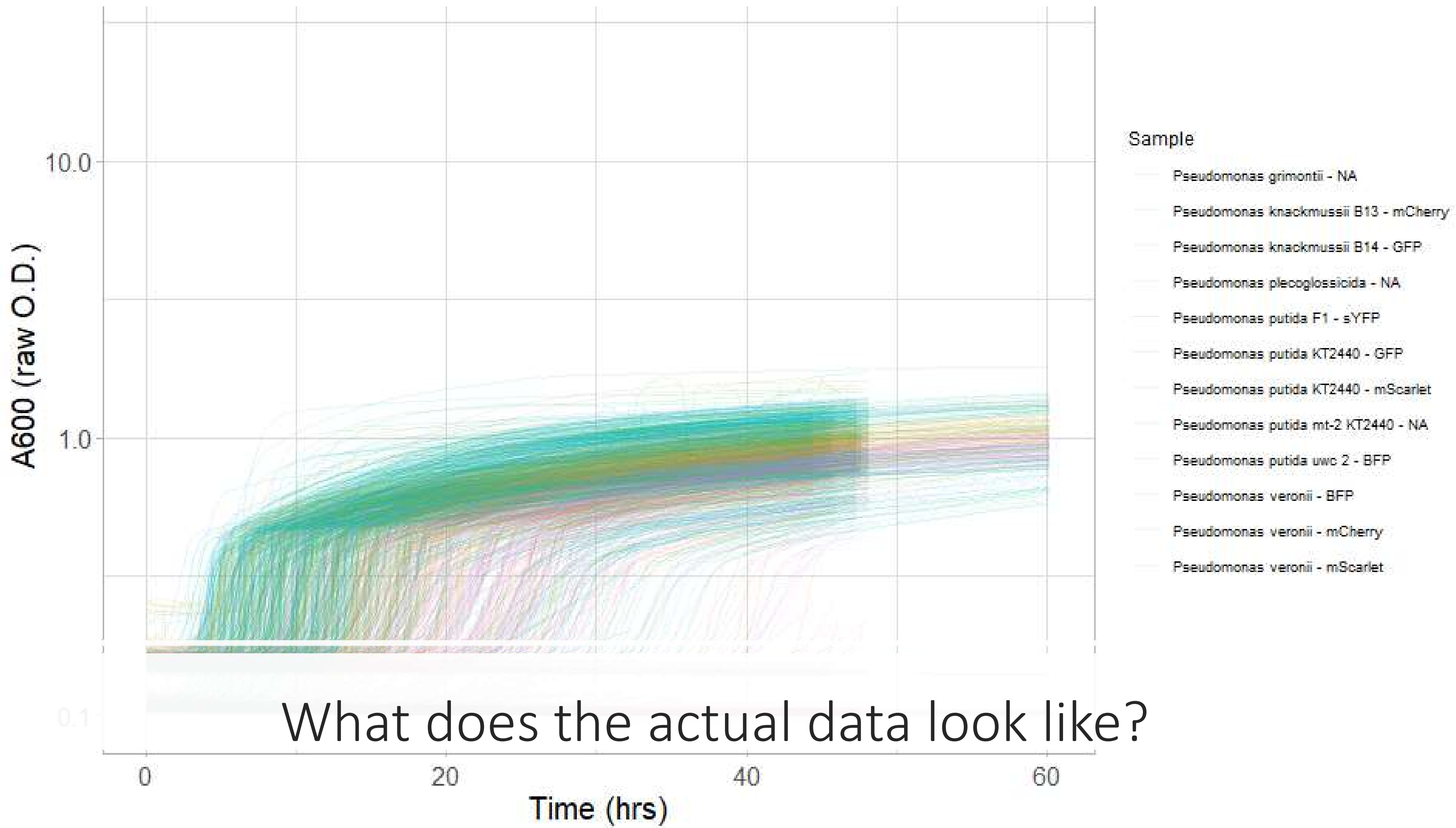


The edge effect: A global problem. The trouble with culturing cells in 96-well plates. Safrany et al., 2021 https://doi.org/10.1016/j.bbrep.2021.100987

LAYOUT	1	2	3	4	5	6	7	8	9	10	11	12
01	2	3	1	5	8	9	6	10	4	7	15	19
02	6	4	10	7	15	19	2	1	3	5	8	9
03	5	8	9	2	3	1	7	19	15	6	4	10

This effect can extend in as far as **three rows** on a 96-well plate.

Same colours spaced out to avoid capturing signals from the adjacent wells



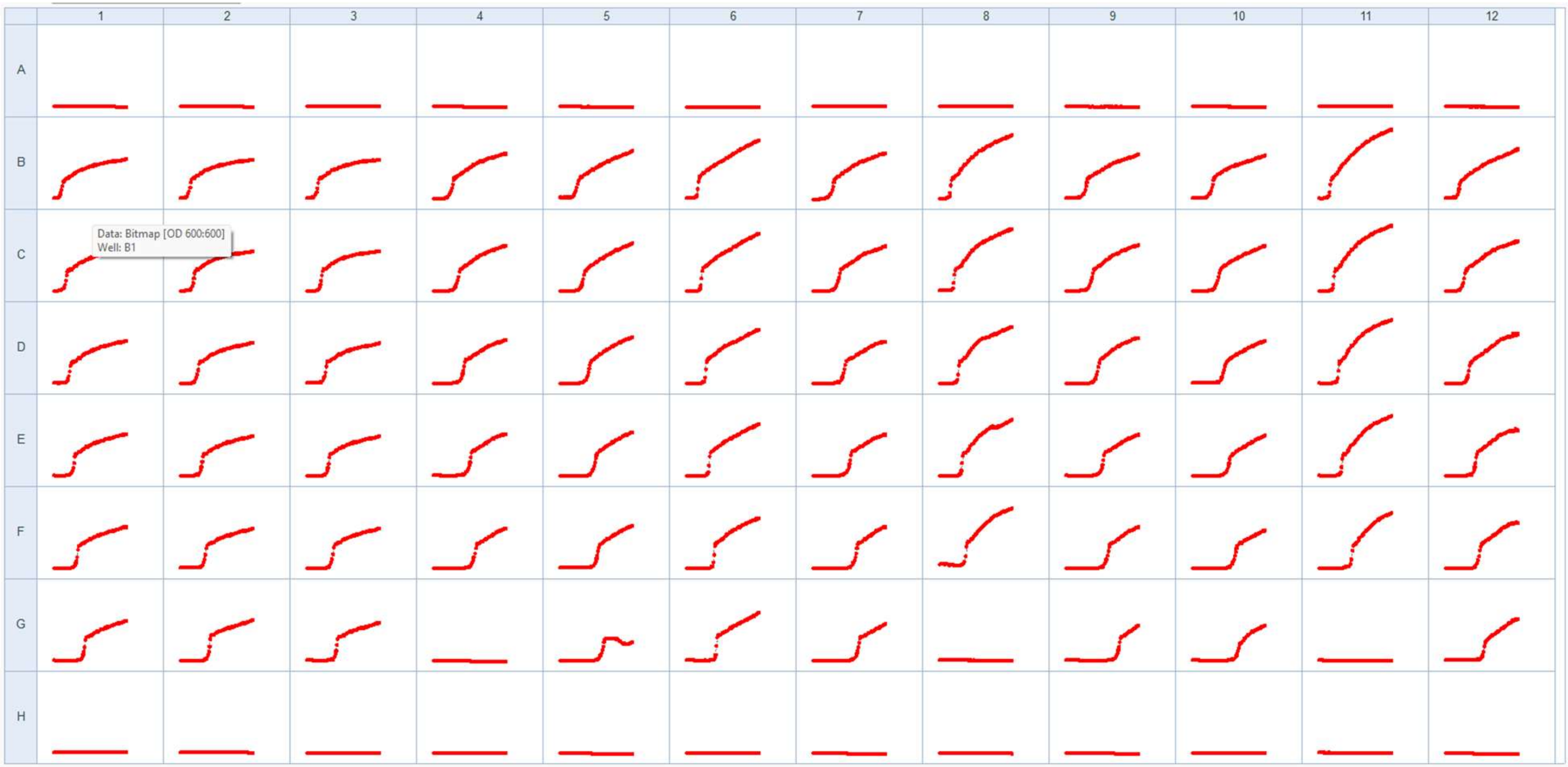
What does the actual data look like?

At 25C

All the strains grew well, even at lower dilutions.

01	2	3	1	5	8	9	6	10	4	7	15	19

Dilution
E-1
E-2
E-3
E-4
E-5
E-6

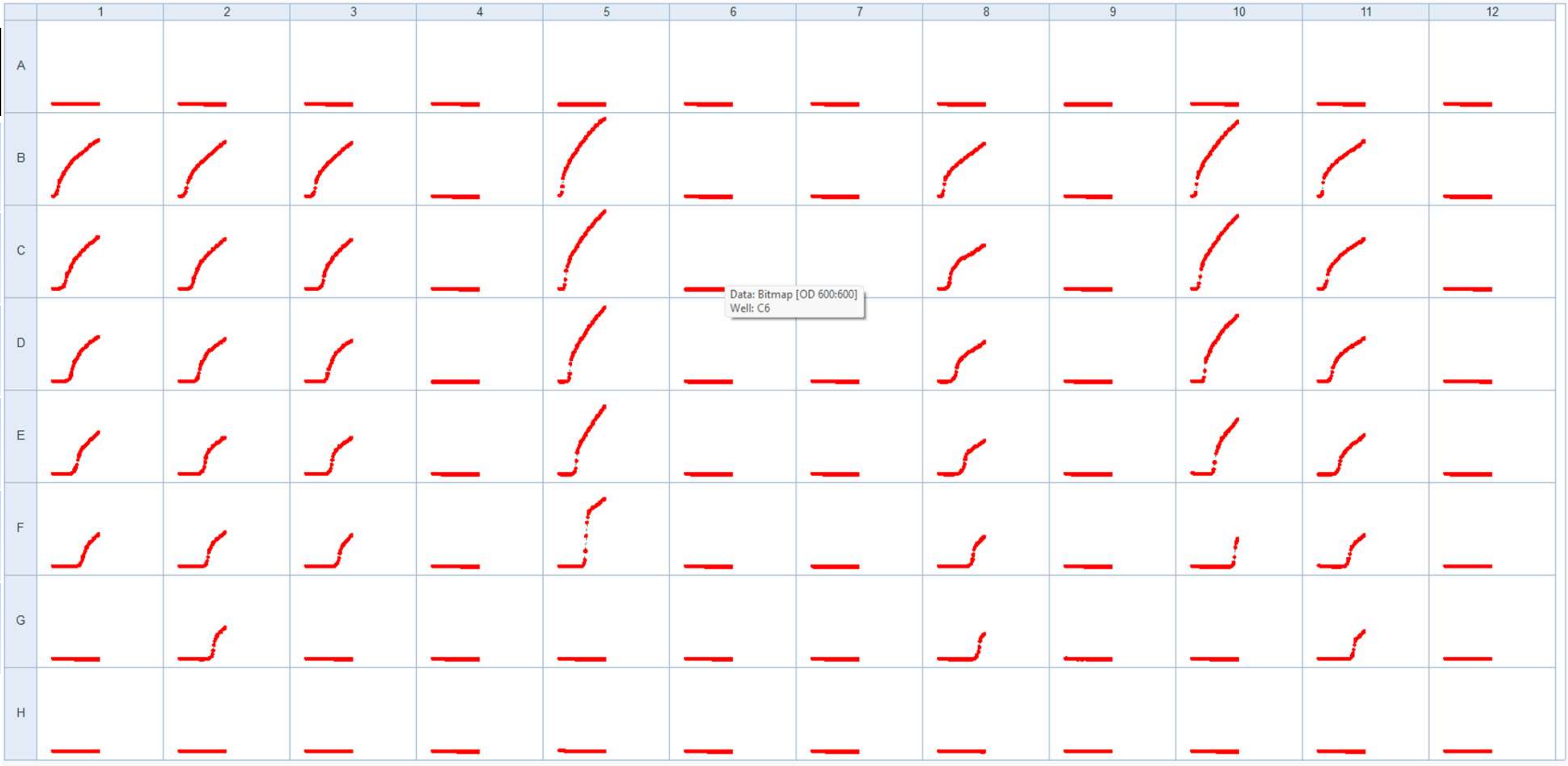


At 40C

Strain 5, 9, 6, 4 and 19 didn’t grow at 40C – less tolerant OR slower acclimatizer.

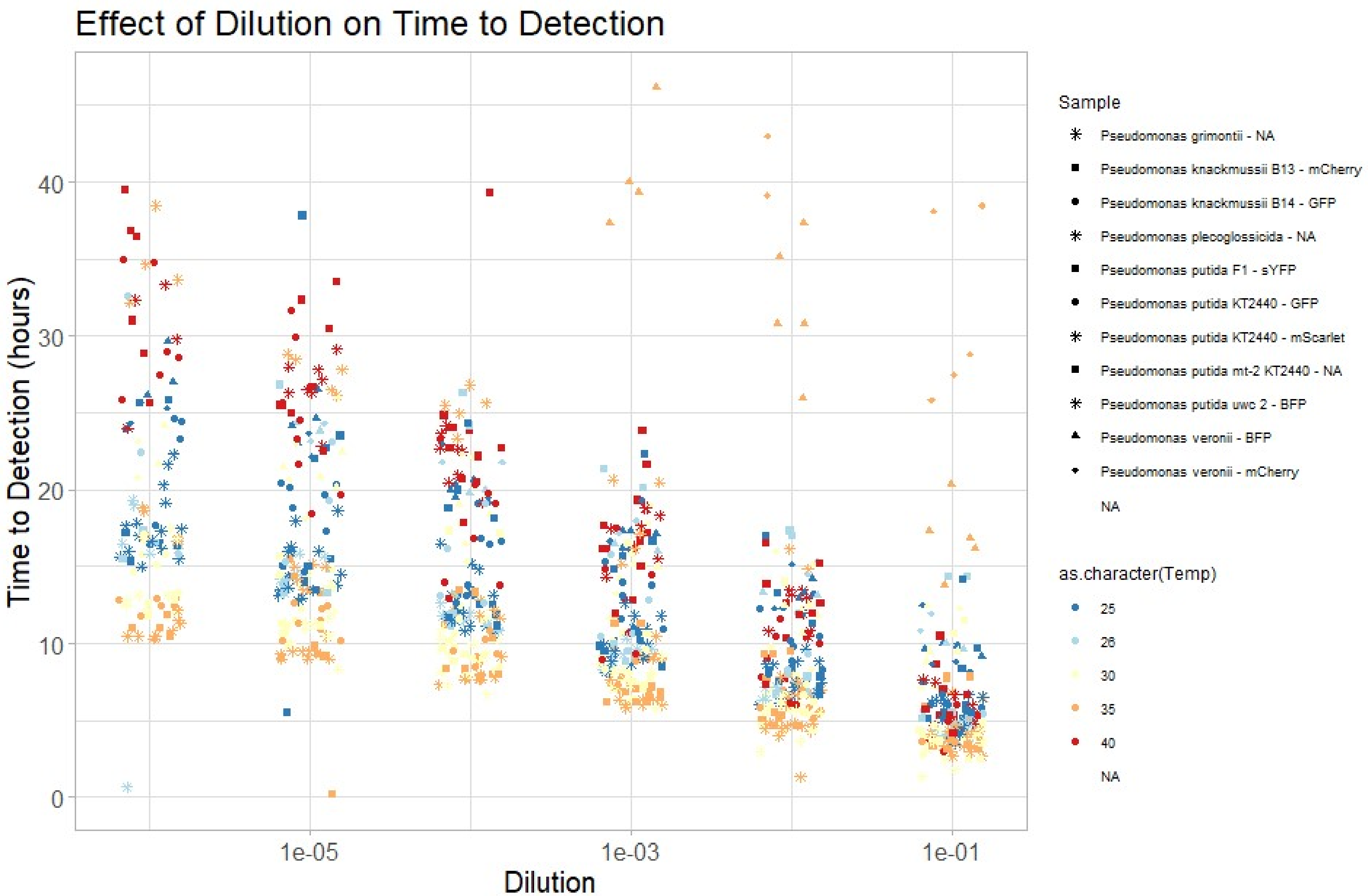
01	2	3	1	5	8	9	6	10	4	7	15	19

Dilution
E-1
E-2
E-3
E-4
E-5
E-6



We can use the Flow Cytometer to check if these strains were alive at 40C

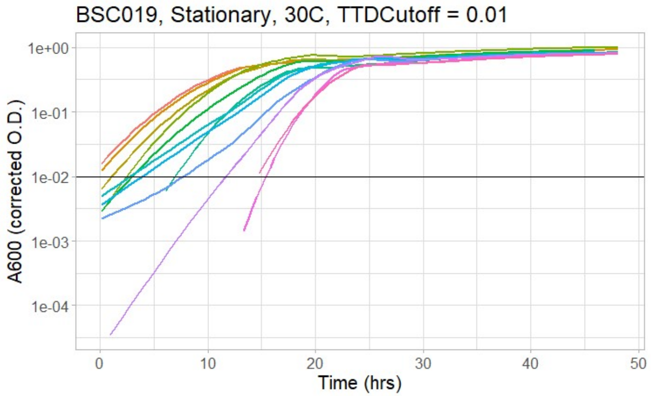
Time-To-Detection (TTD)



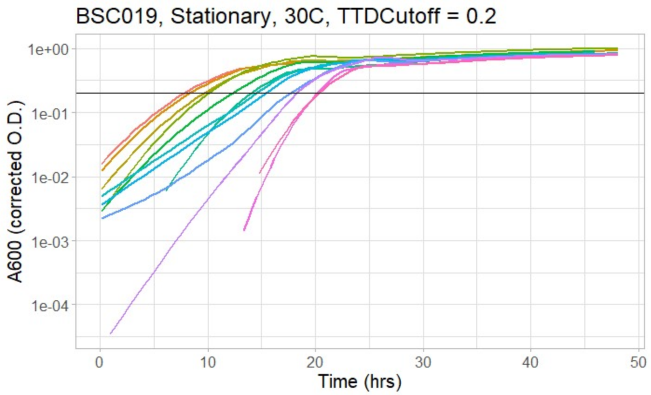
$$t_D = \lambda + \frac{\log N_D - \log N_0}{\mu_m}$$

Growth curve prediction from optical density data.
Mytilinaios et. al., 2011
https://doi.org/10.1016/j.ijfoodmicro.2011.12.035

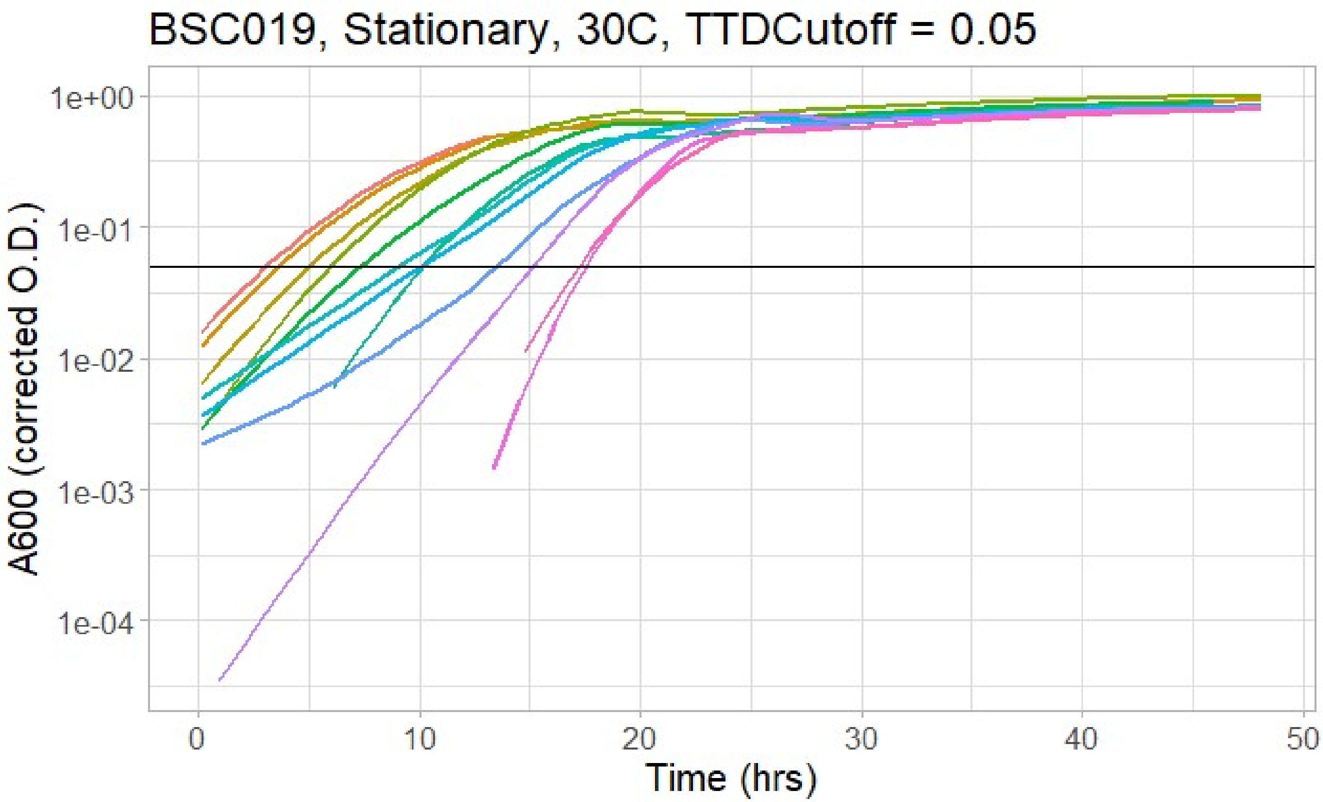
TTD



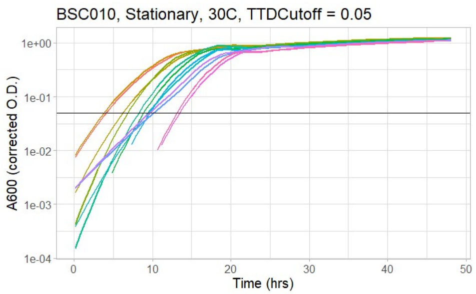
BSC019, Stationary, 30C, TTDCutoff = 0.01
A600 (corrected O.D.)
1e+00
1e-01
1e-02
1e-03
1e-04
0
10
20
30
40
50
Time (hrs)

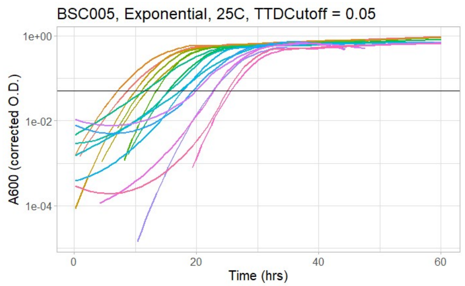


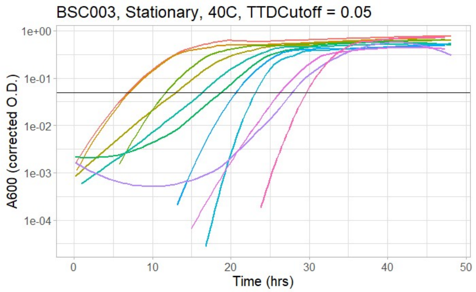
BSC019, Stationary, 30C, TTDCutoff = 0.2
A600 (corrected O.D.)
1e+00
1e-01
1e-02
1e-03
1e-04
0
10
20
30
40
50
Time (hrs)

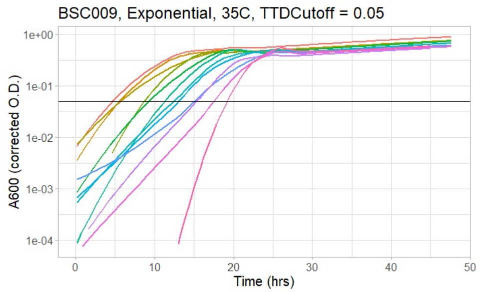


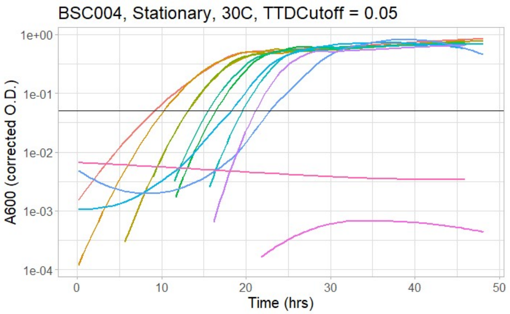
BSC019, Stationary, 30C, TTDCutoff = 0.05
A600 (corrected O.D.)
1e+00
1e-01
1e-02
1e-03
1e-04
0
10
20
30
40
50
Time (hrs)

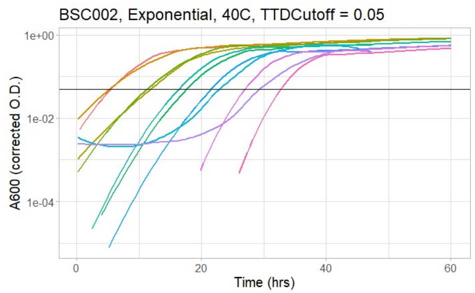

BSC010, Stationary, 30C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)


BSC005, Exponential, 25C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)


BSC003, Stationary, 40C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)


BSC009, Exponential, 35C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)


BSC004, Stationary, 30C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)


BSC002, Exponential, 40C, TTDCutoff = 0.05
A600 (corrected O.D.)
Time (hrs)

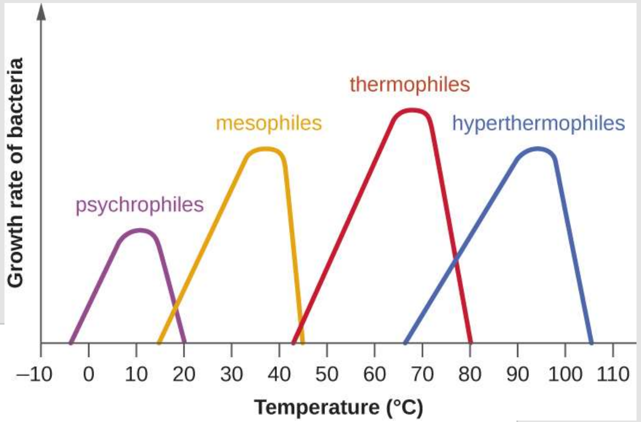
TTD = 0.05

Ranking

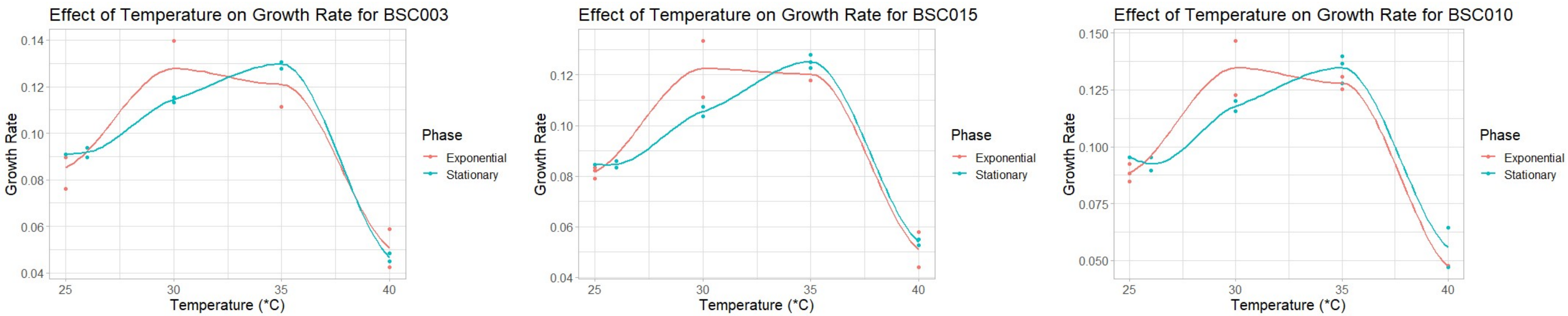
	Phase	Sample	Temp	mean_mu
1	Exponential	Pseudomonas putida KT2440 - mScarlet	30	0.28319749
2	Exponential	Pseudomonas putida uwc 2 - BFP	30	0.23087691
3	Exponential	Pseudomonas putida F1 - sYFP	30	0.19091220
4	Exponential	Pseudomonas putida mt-2 KT2440 - NA	30	0.17690418
5	Exponential	Pseudomonas plecoglossicida - NA	30	0.16705336
6	Exponential	Pseudomonas grimontii - NA	30	0.15509726
7	Exponential	Pseudomonas knackmussii B14 - GFP	30	0.15224437
8	Exponential	Pseudomonas knackmussii B13 - mCherry	30	0.12527195
9	Exponential	Pseudomonas veronii - mScarlet	30	0.12527195
10	Exponential	Pseudomonas veronii - mCherry	30	0.11101954
11	Exponential	Pseudomonas veronii - BFP	30	0.08923929
12	Exponential	Pseudomonas putida KT2440 - GFP	30	NaN

	Phase	Sample	mean_mu
1	Stationary	Pseudomonas putida F1 - sYFP	0.19860193
2	Stationary	Pseudomonas putida uwc 2 - BFP	0.18227848
3	Stationary	Pseudomonas putida KT2440 - mScarlet	0.17959133
4	Stationary	Pseudomonas putida mt-2 KT2440 - NA	0.16718296
5	Stationary	Pseudomonas grimontii - NA	0.16503973
6	Stationary	Pseudomonas putida KT2440 - GFP	0.15031315
7	Stationary	Pseudomonas knackmussii B14 - GFP	0.13662239
8	Stationary	Pseudomonas veronii - BFP	0.08118735
9	Stationary	Pseudomonas veronii - mScarlet	0.07633600
10	Stationary	Pseudomonas knackmussii B13 - mCherry	0.07579425
11	Stationary	Pseudomonas veronii - mCherry	0.06427440
12	Stationary	Pseudomonas plecoglossicida - NA	NaN

Thermal performance curves



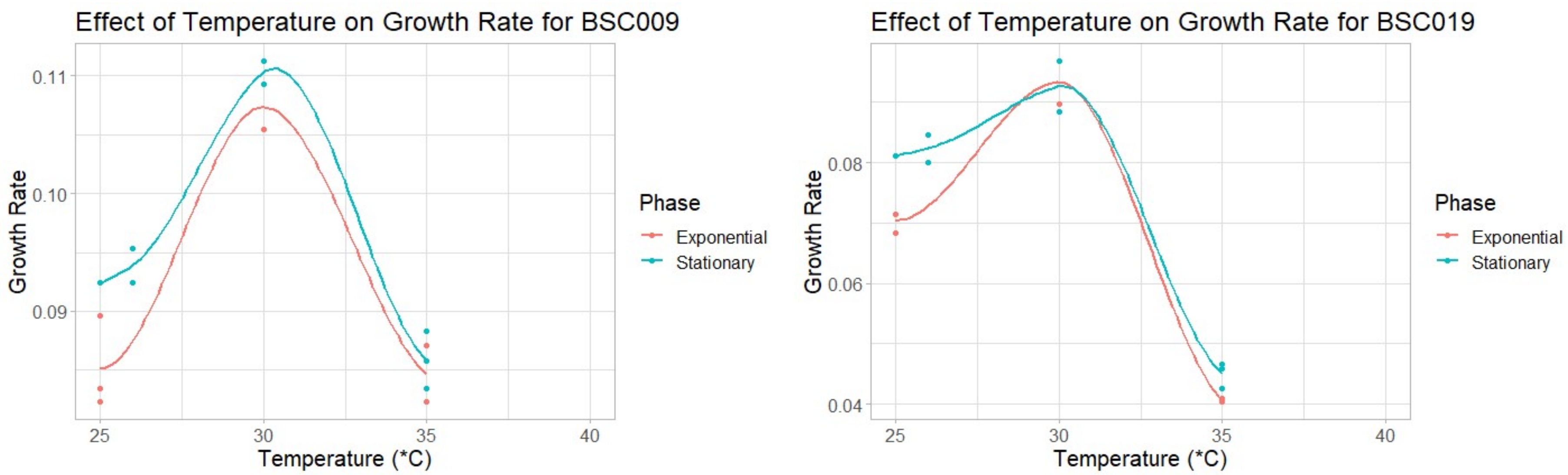
Source



Optimum temperature shifting rightwards

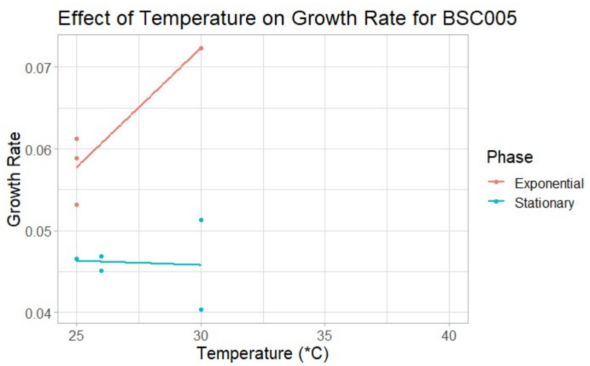
*Growth Rate = 1/TTD
Coarse-grained analysis

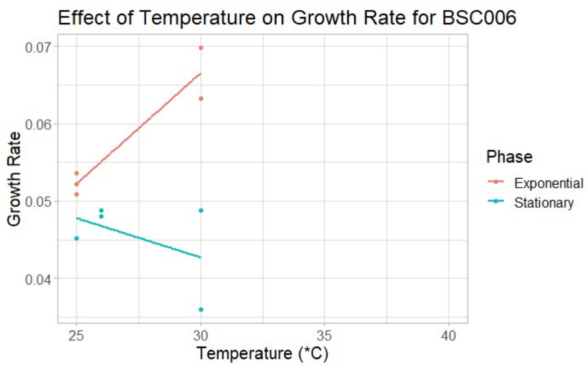
TPCs

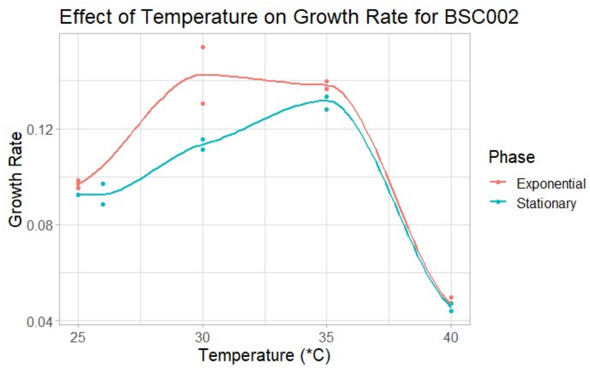


Optimum temperature remains same

TPCs



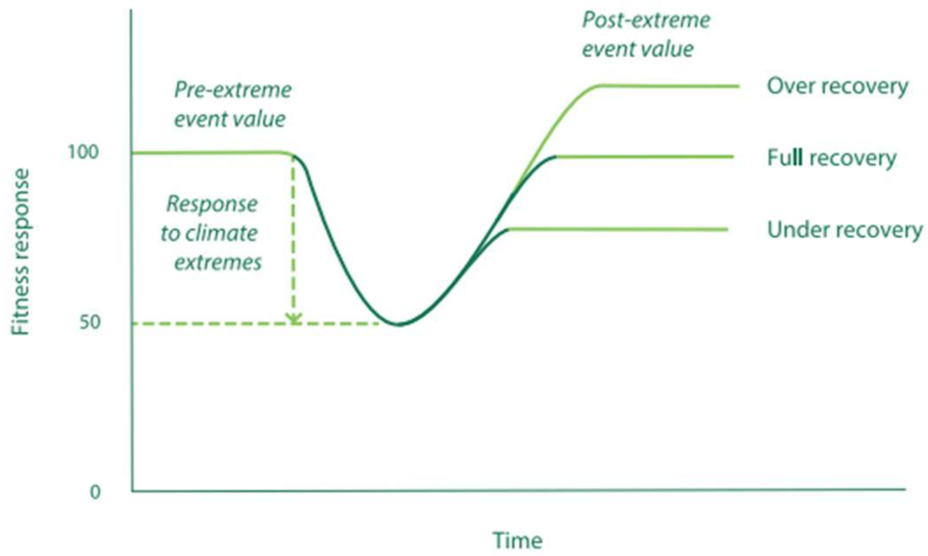




Need more data points at lower temperatures

Resistance

- $Resistance = \frac{OD_{T°C}}{OD_{25°C}}$



Resilience of rhizosphere microbial predators and their prey communities after an extreme heat event. Thakur et. al., 2020

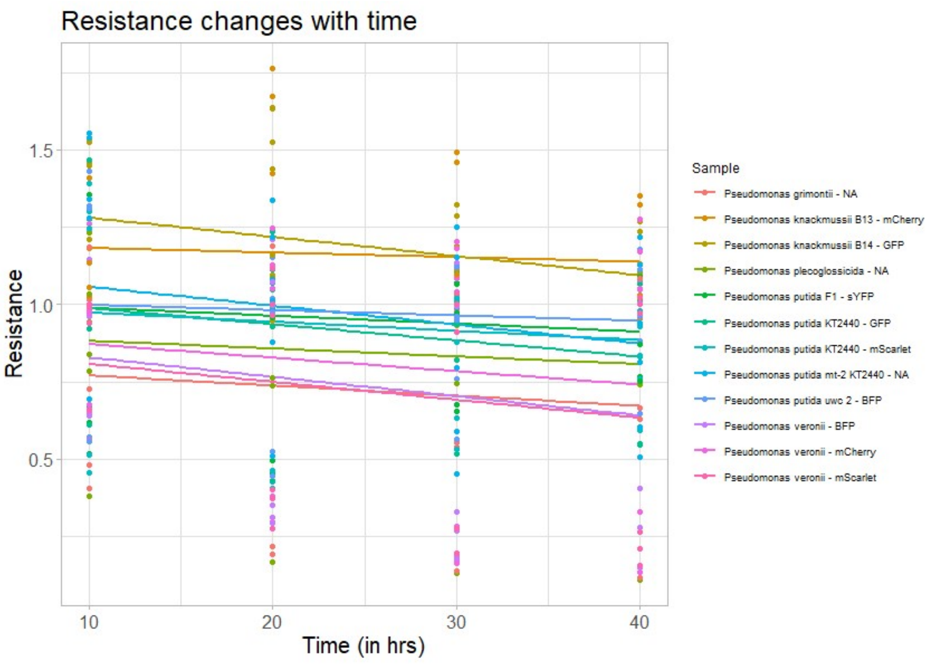
Biotic responses to climate extremes in terrestrial ecosystems. Thakur et. al., 2022

Ranking

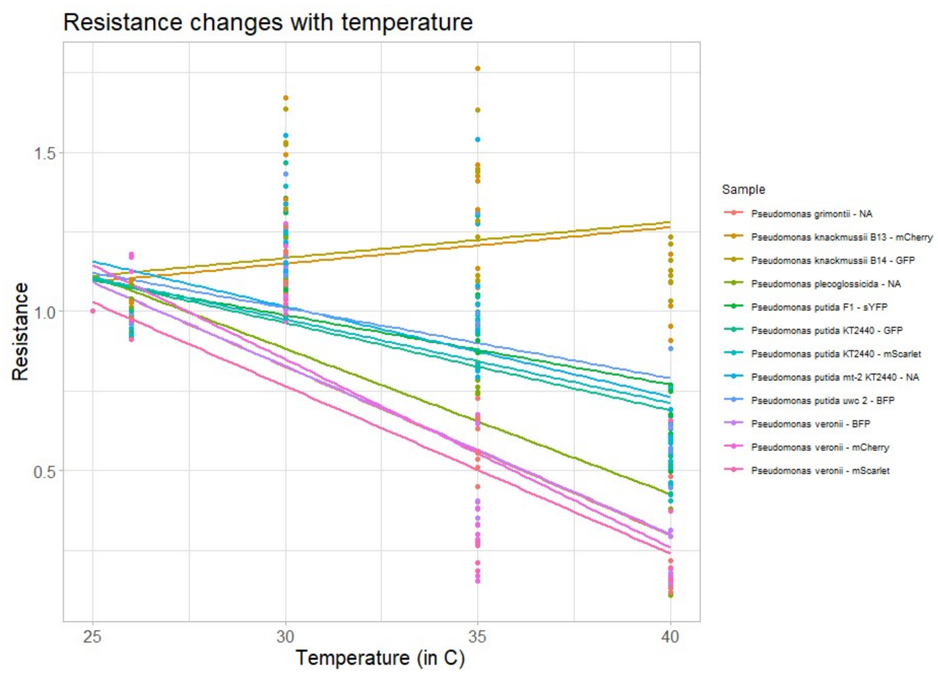
	Phase	Sample	Temp	Resistance_after_9.99
1	Exponential	Pseudomonas knackmussii B14 - GFP	35	1.4610610
2	Exponential	Pseudomonas putida uwc 2 - BFP	35	1.2350027
3	Exponential	Pseudomonas knackmussii B13 - mCherry	35	1.4093879
4	Exponential	Pseudomonas putida F1 - sYFP	35	1.2763520
5	Exponential	Pseudomonas putida KT2440 - mScarlet	35	1.2339901
6	Exponential	Pseudomonas putida KT2440 - GFP	35	1.2743298
7	Exponential	Pseudomonas putida mt-2 KT2440 - NA	35	1.2795301
8	Exponential	Pseudomonas plecoglossicida - NA	35	0.7859160
9	Exponential	Pseudomonas grimontii - NA	35	0.6548512
10	Exponential	Pseudomonas veronii - BFP	35	0.6617025
11	Exponential	Pseudomonas veronii - mScarlet	35	0.5625164
12	Exponential	Pseudomonas veronii - mCherry	35	0.6471403

	Phase	Sample	Temp	Resistance_after_9.99
1	Stationary	Pseudomonas putida mt-2 KT2440 - NA	35	1.5811456
2	Stationary	Pseudomonas knackmussii B14 - GFP	35	1.5032668
3	Stationary	Pseudomonas putida KT2440 - GFP	35	1.3854287
4	Stationary	Pseudomonas putida F1 - sYFP	35	1.3174784
5	Stationary	Pseudomonas putida uwc 2 - BFP	35	1.3136008
6	Stationary	Pseudomonas putida KT2440 - mScarlet	35	1.3024147
7	Stationary	Pseudomonas knackmussii B13 - mCherry	35	1.1154446
8	Stationary	Pseudomonas plecoglossicida - NA	35	0.8200244
9	Stationary	Pseudomonas grimontii - NA	35	0.7198012
10	Stationary	Pseudomonas veronii - mScarlet	35	0.6869152
11	Stationary	Pseudomonas veronii - mCherry	35	0.6867805
12	Stationary	Pseudomonas veronii - BFP	35	0.6722269

Resistance

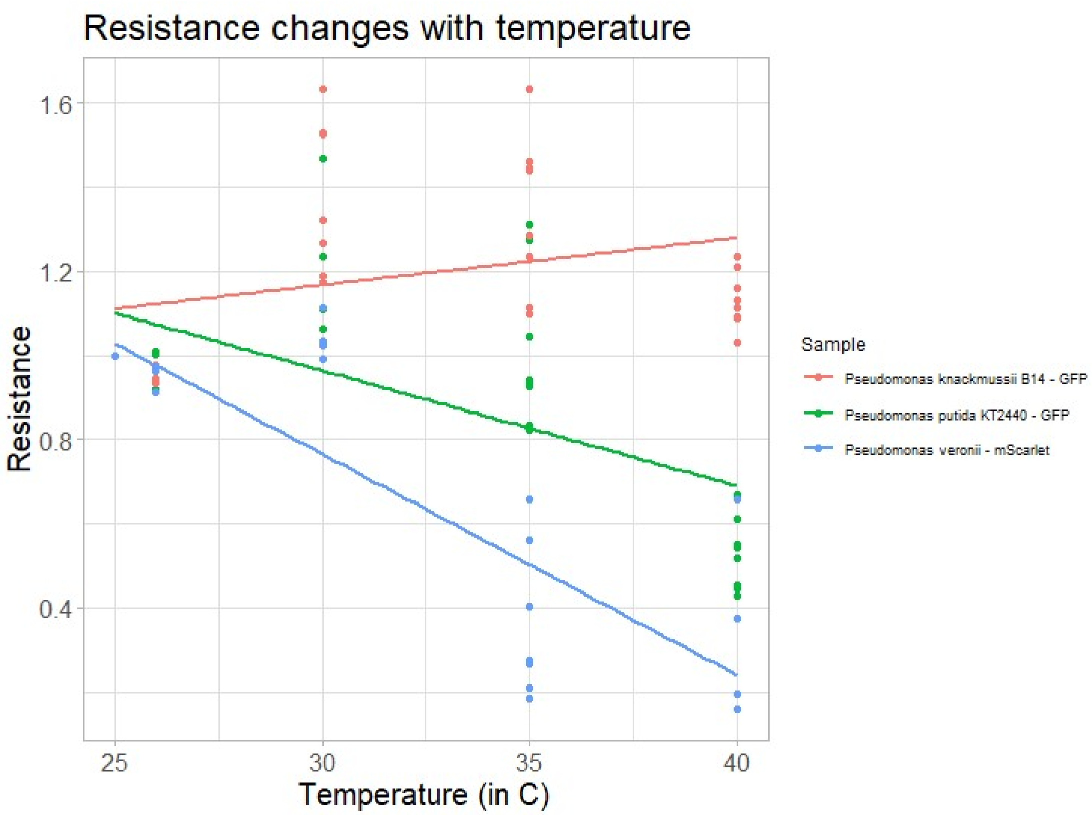


Resistance changes with time
Resistance
1.5
1.0
0.5
10
20
30
40
Time (in hrs)
Sample
Pseudomonas grimontii - NA
Pseudomonas knackmussii B13 - mCherry
Pseudomonas knackmussii B14 - GFP
Pseudomonas plecoglossicida - NA
Pseudomonas putida F1 - sYFP
Pseudomonas putida KT2440 - GFP
Pseudomonas putida KT2440 - mScarlet
Pseudomonas putida mt-2 KT2440 - NA
Pseudomonas putida uwc 2 - BFP
Pseudomonas veronii - BFP
Pseudomonas veronii - mCherry
Pseudomonas veronii - mScarlet

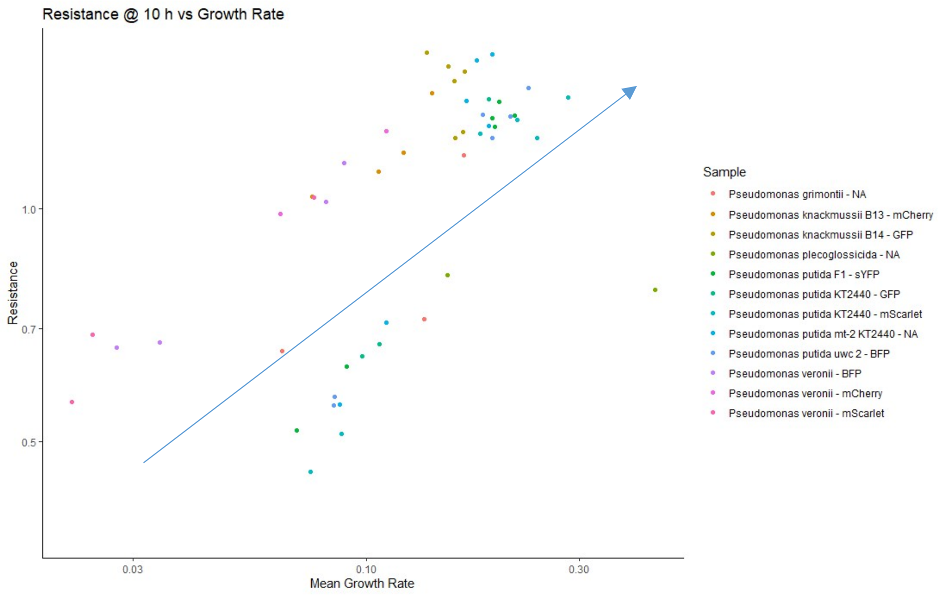


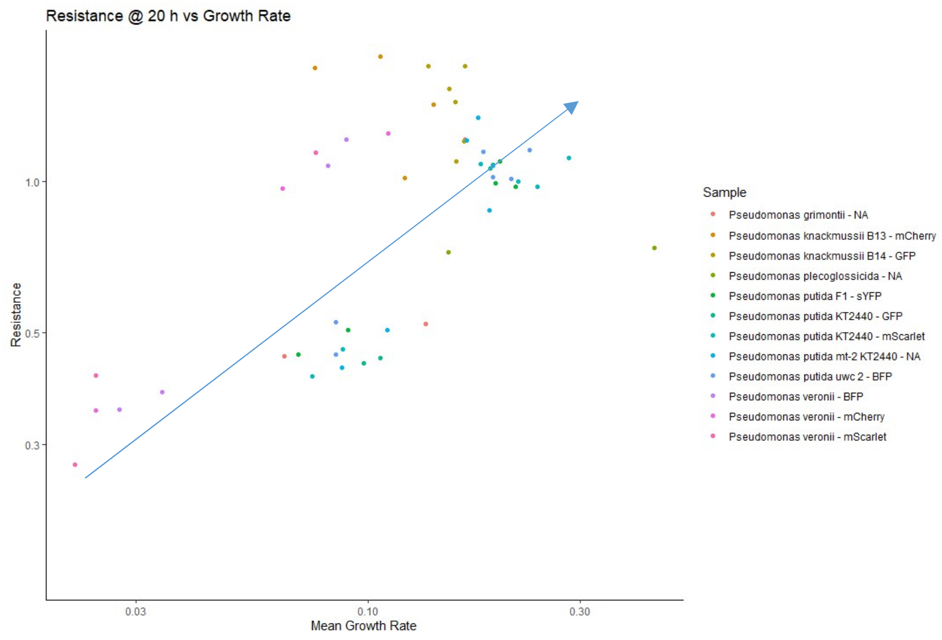
Resistance changes with temperature
Resistance
1.5
1.0
0.5
25
30
35
40
Temperature (in C)
Sample
Pseudomonas grimontii - NA
Pseudomonas knackmussii B13 - mCherry
Pseudomonas knackmussii B14 - GFP
Pseudomonas plecoglossicida - NA
Pseudomonas putida F1 - sYFP
Pseudomonas putida KT2440 - GFP
Pseudomonas putida KT2440 - mScarlet
Pseudomonas putida mt-2 KT2440 - NA
Pseudomonas putida uwc 2 - BFP
Pseudomonas veronii - BFP
Pseudomonas veronii - mCherry
Pseudomonas veronii - mScarlet

Resistance

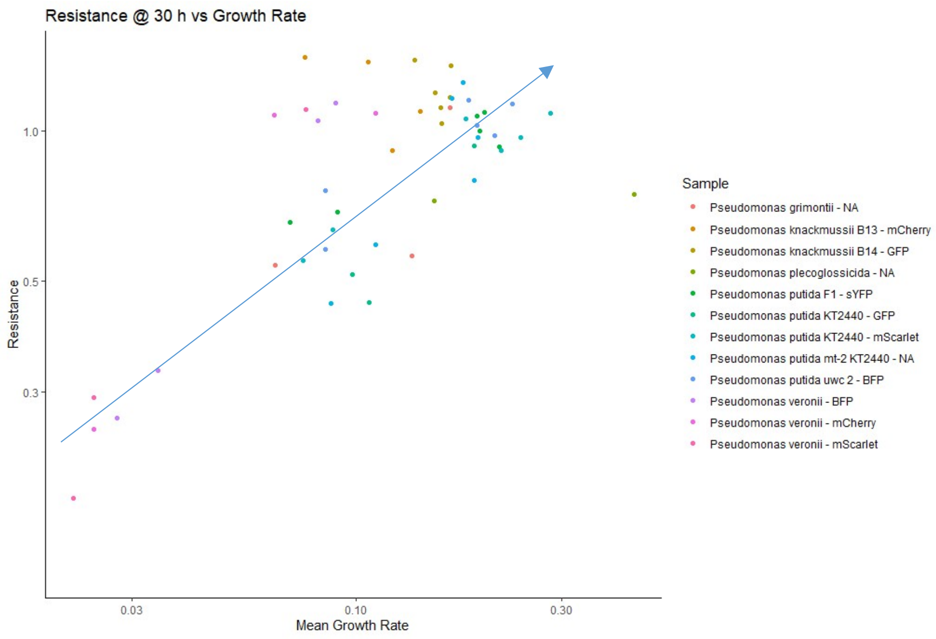

Resistance changes with temperature
1.6
1.2
0.8
0.4
Resistance
25
30
35
40
Temperature (in C)
Sample
Pseudomonas knackmussii B14 - GFP
Pseudomonas putida KT2440 - GFP
Pseudomonas veronii - mScarlet

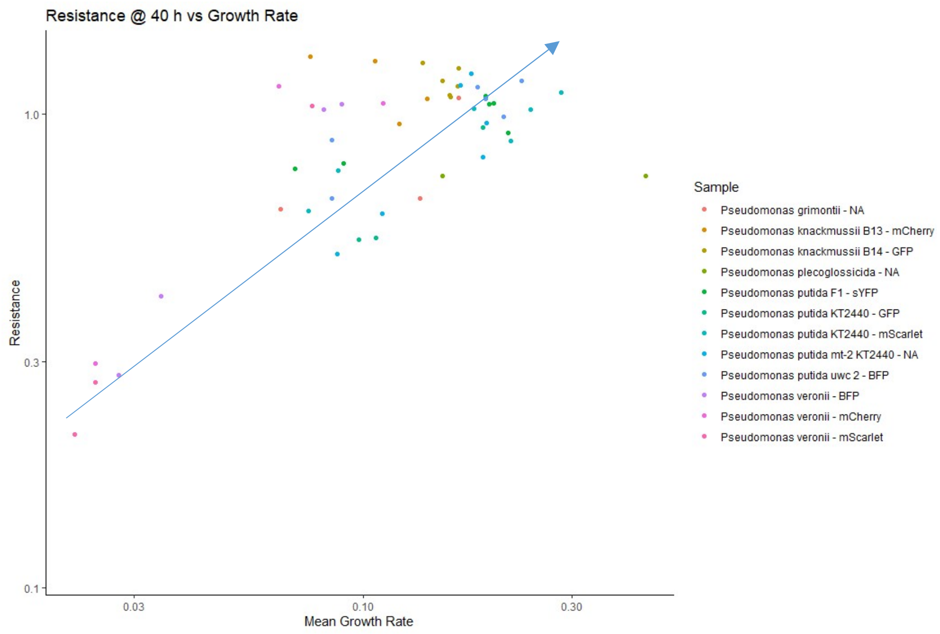
Resistance vs Growth Rate



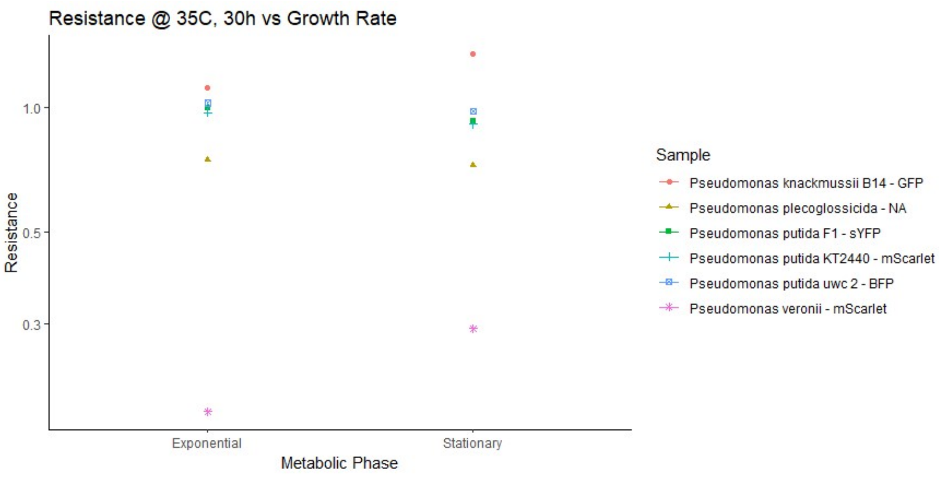


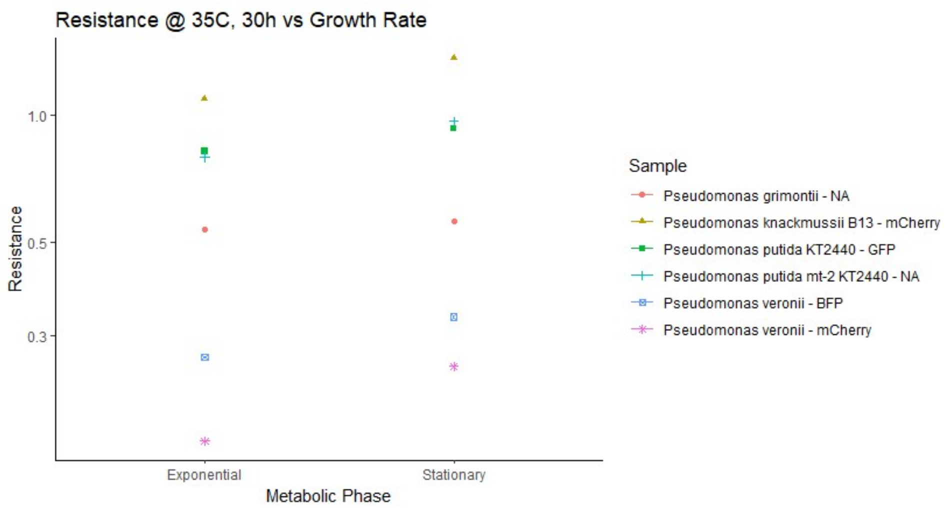
Resistance vs Growth Rate



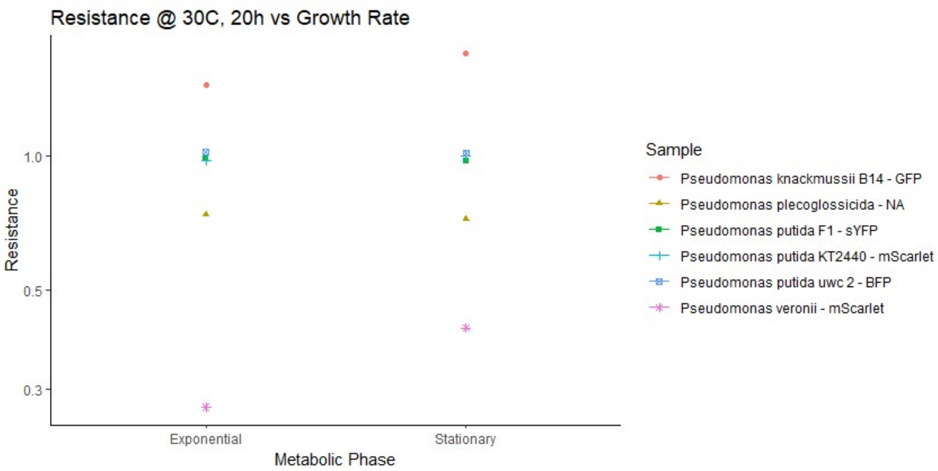


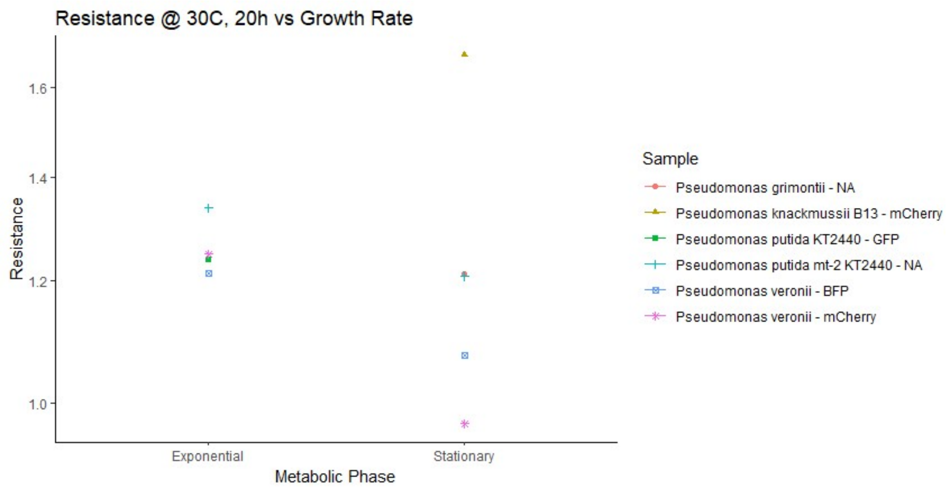
Resistance vs Metabolic Phase



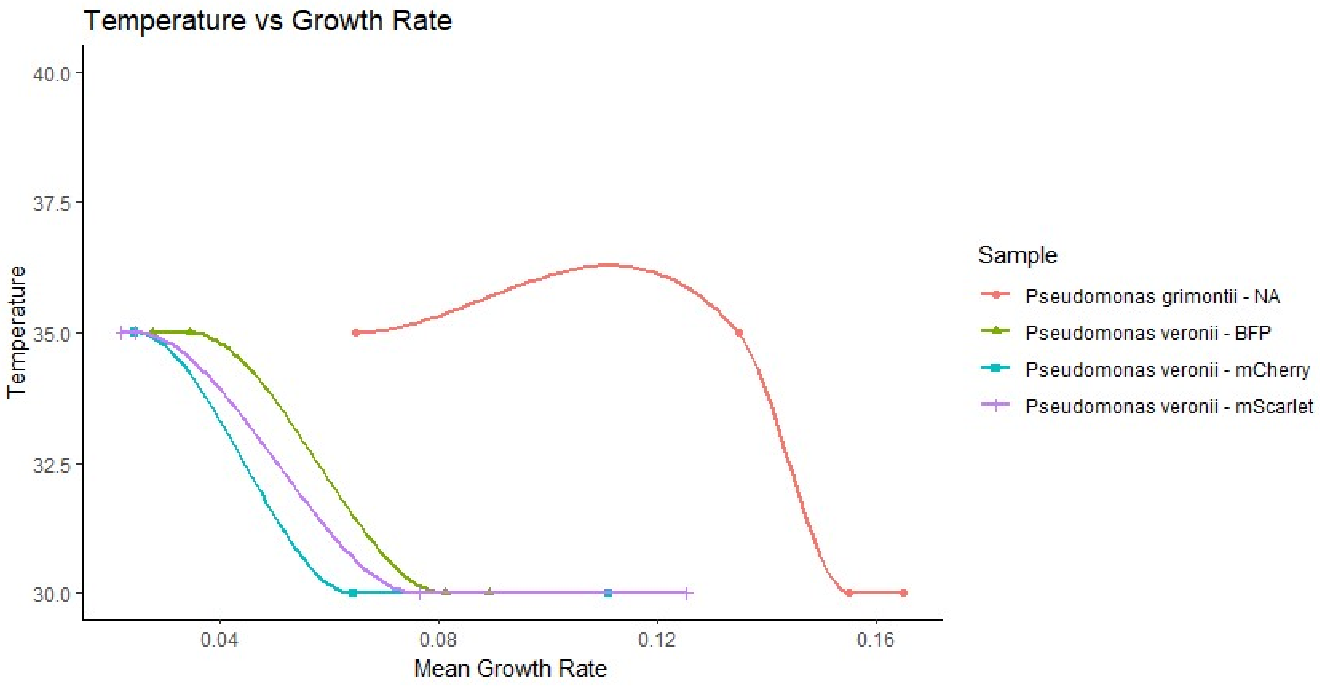


Resistance vs Metabolic Phase



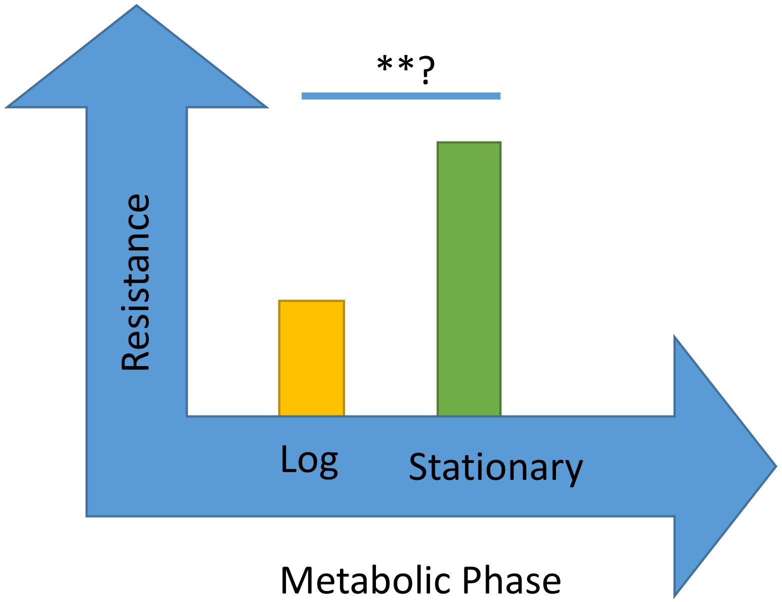


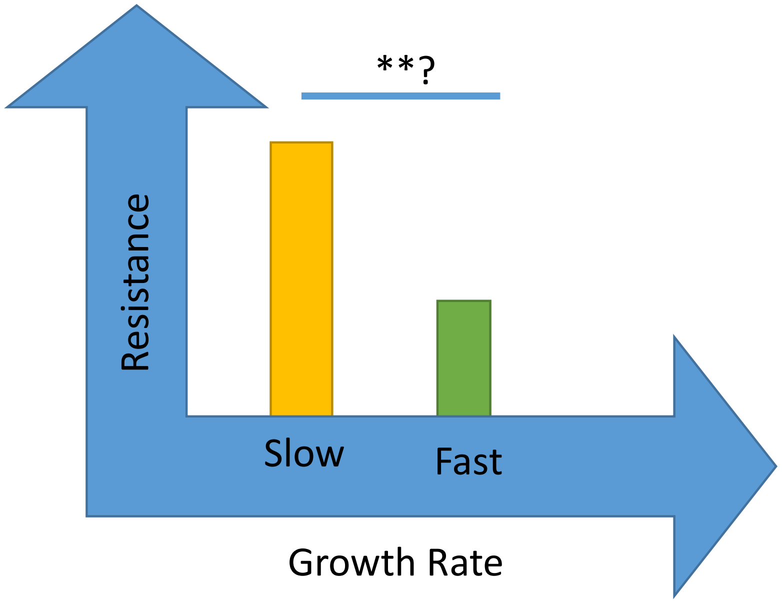
Temperature vs Growth Rate



Hypothesis – accepted/rejected?

not yet a . rather ...





Future Directions

- A more fine-grained analysis of the collected data.
- From relative number (dilutions) to absolute number (CFU/mL) at different temperatures – to check how many cells do we start with.
- Calibrating the plate reader (from OD to CFU/mL)
  - Also, crucial to find the saturation limit beyond which OD isn't a reliable indicator of cell number.