



**PPI**  
PREDICTIVE PHENOMICS  
INITIATIVE  
@PNNL

# Microbial community engineering to reduce carbon and nitrogen footprints in biomanufacturing

March 4, 2024

**Pavlo Bohutskyi, Kyle Pomraning**

U.S. DEPARTMENT OF  
**ENERGY** **BATTELLE**

PNNL is operated by Battelle for the U.S. Department of Energy



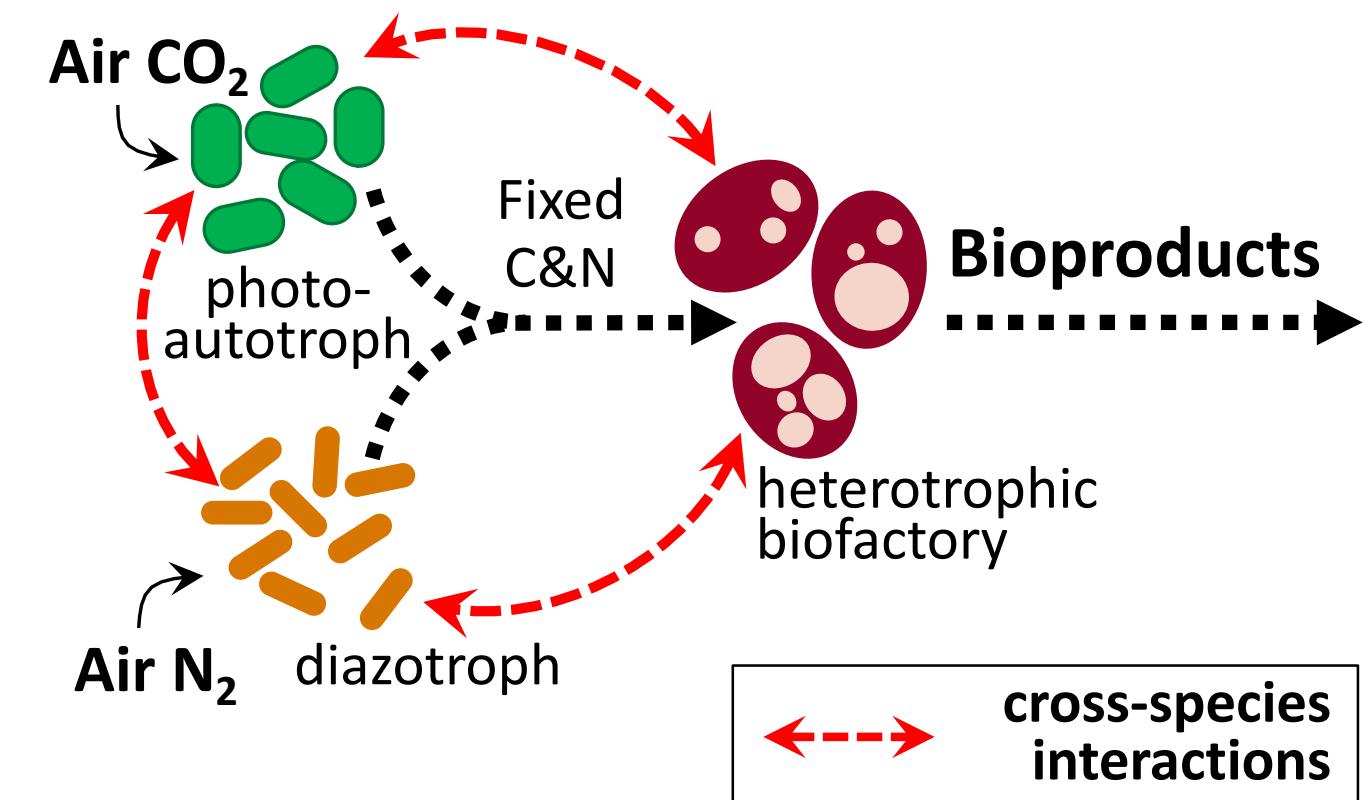
# Project Goals and Impact

**Vision:** Enable rational design and control of novel bioproduction platforms based on microbial communities to facilitate reduced carbon and nitrogen footprints

**Impact:** Reduce GHG emissions in biomanufacturing by *in situ* fixation of C & N

## Two main project goals:

- I. Establish and characterize model microbial communities for bioproduction using  $\text{CO}_2$  and  $\text{N}_2$
- II. Enable phenotype prediction to allow rational phenotype design and control



## Phenotype 2: Enhanced cyanobacteria fitness under stress conditions



### Task 2. Increase bio-production by improving fitness under stress conditions (robustness)

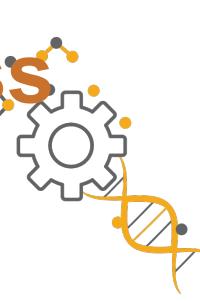
**Motivation:** Transfer of laboratory strains into industrial environment fails often due to its reduction of strains fitness under stress conditions

**Hypothesis (cyanobacteria).** Stress conditions negatively impact the fitness (viability, growth) and bio-production of the biocatalysts. Fitness and bio-production can be in part recovered through adaptation and through interactions with the co-culture partner.

**Phenotype (cyanobacteria):** Culture fitness (viability, growth rate) and bio-production rate.

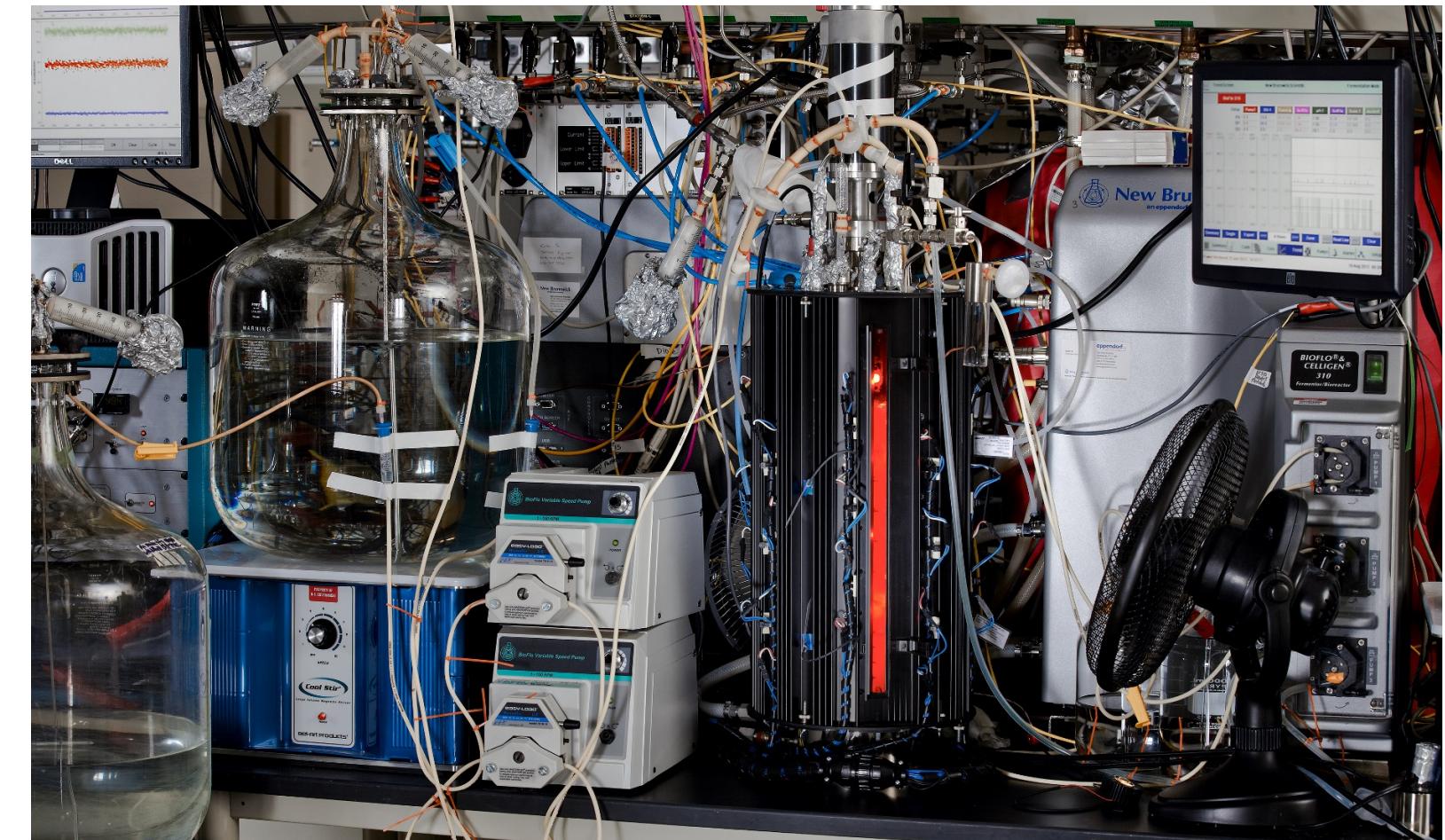
**Goals:** Reveal mechanisms for fitness and bio-production reduction under stress; and improve fitness and bioproduction under stress.

# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions



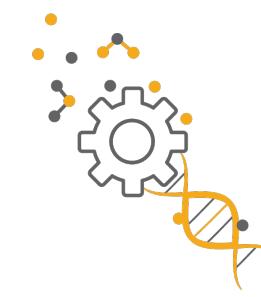
## Applied approach:

- operate photobioreactor as turbidostat (constant density of the culture)
- continuously increase the oxidative stress resistance
- transcriptome samples to characterize cellular response to stress
- select cells of highest fitness under stress (directed evolution)
- collect stress adapted cell-lines of a cyanobacterium *S. elongatus*
- transcriptome samples to characterize adapted cell-lines



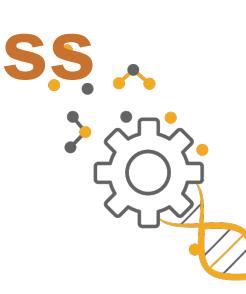
Photobioreactor system (turbidostat mode) for selection of cells with highest fitness (highest growth rate)

# List and description of stress conditions samples

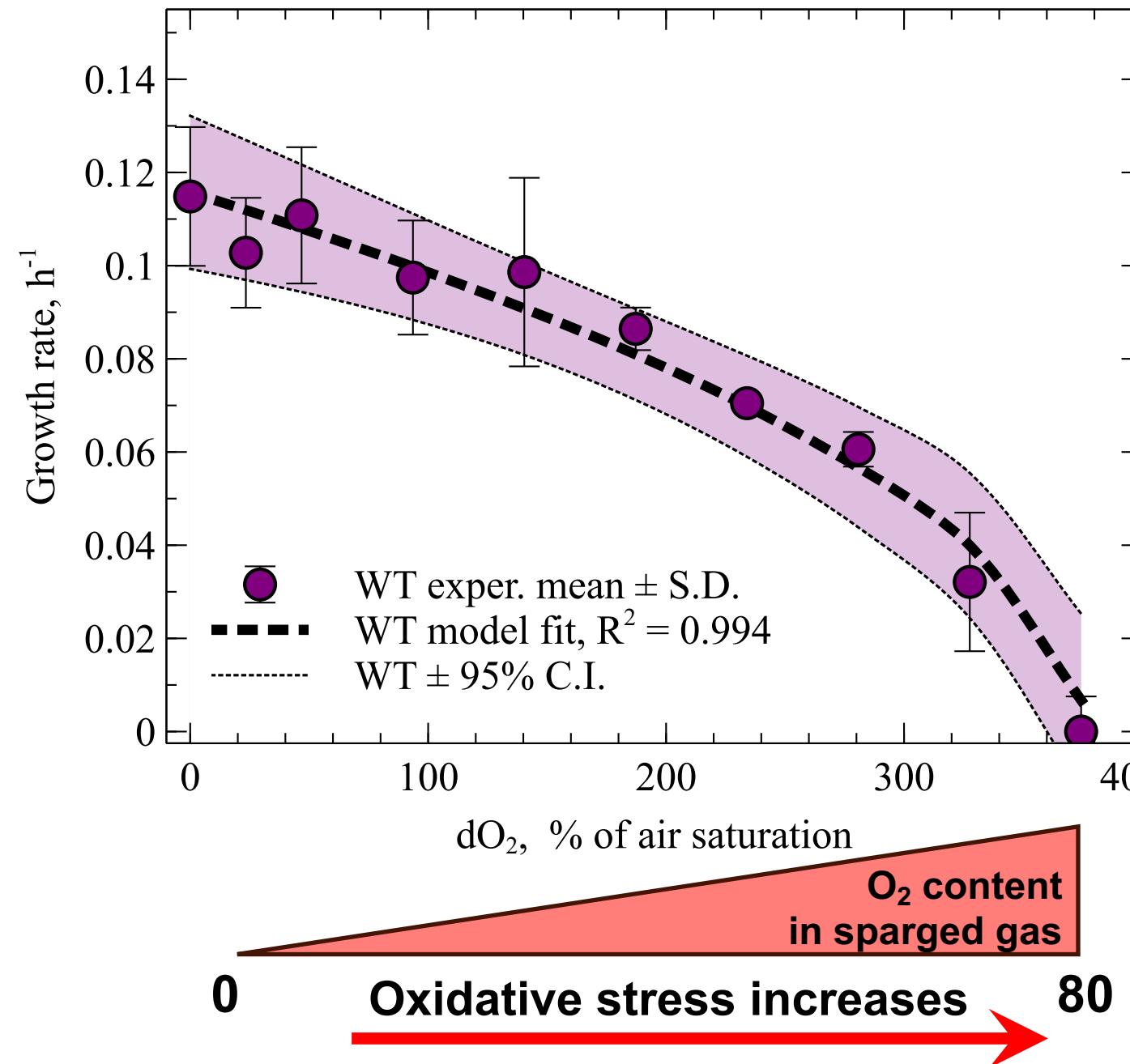


Sample		Gases sparged into PBR, L/min			O <sub>2</sub> in supplied gas, %	Dissolved O <sub>2</sub> from air saturation, %	Light	Steady state sample description	Note	
#	Name	CO <sub>2</sub>	N <sub>2</sub> or *air	O <sub>2</sub>						
1.1	WT100.0A	0.04	2	0	0	0.0	50:50	WT cells under medium light and no O <sub>2</sub> stress	Imposing oxidative stress on WT cyano by increasing O <sub>2</sub>	
1.2	WT100.0B	0.04	2	0	0	0.0	50:50			
2.1	WT205.0A	0.04	2	0	0	0.0	140:65	WT cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
2.2	WT205.0B	0.04	2	0	0	0.0	140:65			
3.1	WT205.5A	0.04	*1.5	0.5	4.9	23.4	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
3.2	WT205.5B	0.04	*1.5	0.5	4.9	23.4	140:65			
4.1	WT205.10A	0.04	1.8	0.2	9.8	46.8	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
4.2	WT205.10B	0.04	1.8	0.2	9.8	46.8	140:65			
5.1	WT205.20A	0.04	1.6	0.4	19.6	93.6	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
5.2	WT205.20B	0.04	1.6	0.4	19.6	93.6	140:65			
6.1	WT205.30A	0.04	1.4	0.6	29.4	140	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
6.2	WT205.30B	0.04	1.4	0.6	29.4	140	140:65			
7.1	WT205.40A	0.04	1.2	0.8	39.2	187	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
7.2	WT205.40B	0.04	1.2	0.8	39.2	187	140:65			
8.1	WT205.50A	0.04	1	1	49.0	234	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
8.2	WT205.50B	0.04	1	1	49.0	234	140:65			
9.1	WT205.60A	0.04	0.8	1.2	58.8	281	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
9.2	WT205.60B	0.04	0.8	1.2	58.8	281	140:65			
10.1	WT205.70A	0.04	0.6	1.4	68.6	328	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
10.2	WT205.70B	0.04	0.6	1.4	68.6	328	140:65			
11.1	WT205.80A	0.04	0.4	1.6	78.4	374	140:65	WT cells under high light and imposed O <sub>2</sub> stress (max growth rate reference)		
11.2	WT205.80B	0.04	0.4	1.6	78.4	374	140:65			

# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions



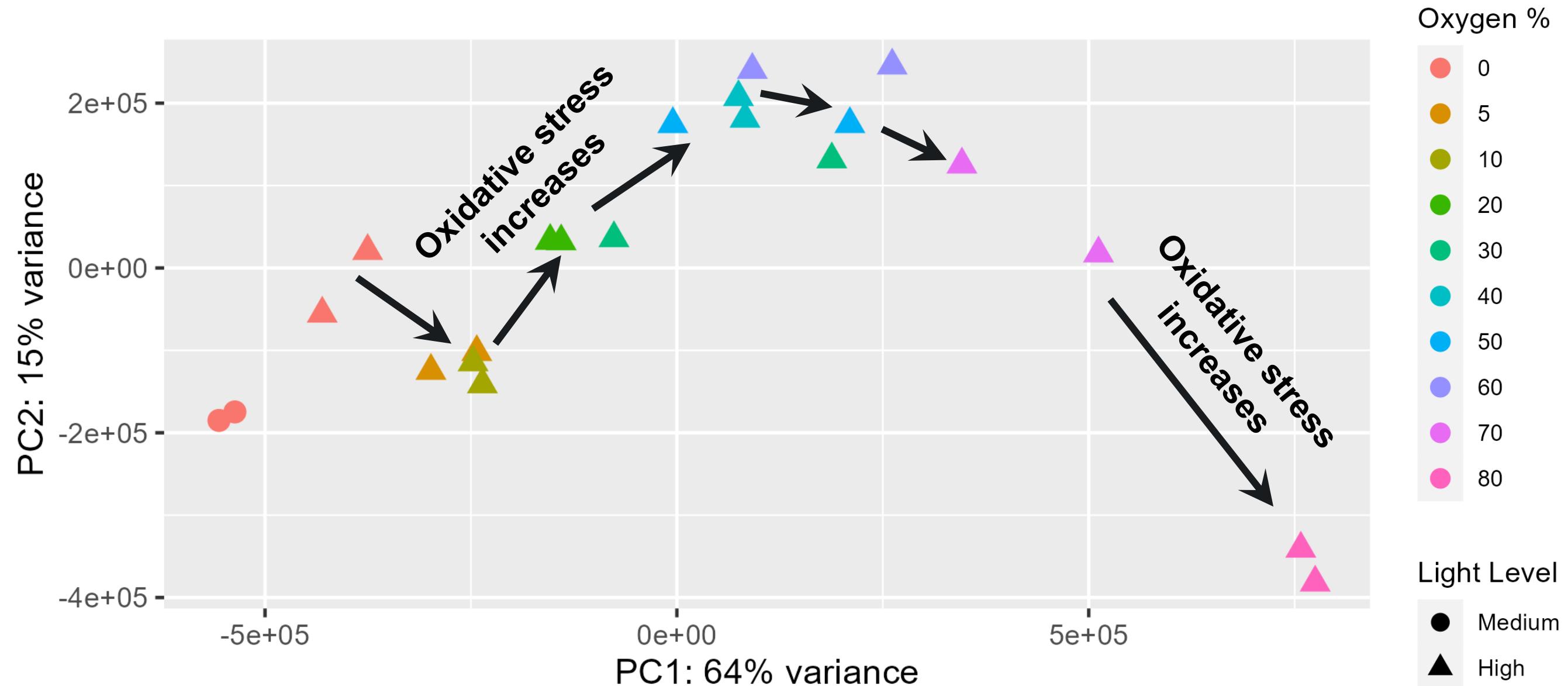
## WT cells growth rate phenotype adjustment in response to oxidative stress



- growth rate of WT cells reduces along with increasing oxidative stress
- WT cells stop growing when  $\text{dO}_2$  reached  $\sim 375$  of air saturation
- culture was maintained at high oxidative stress to select adapted cells

# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

## WT cyanobacteria gene expression adjustment in response of to oxidative stress

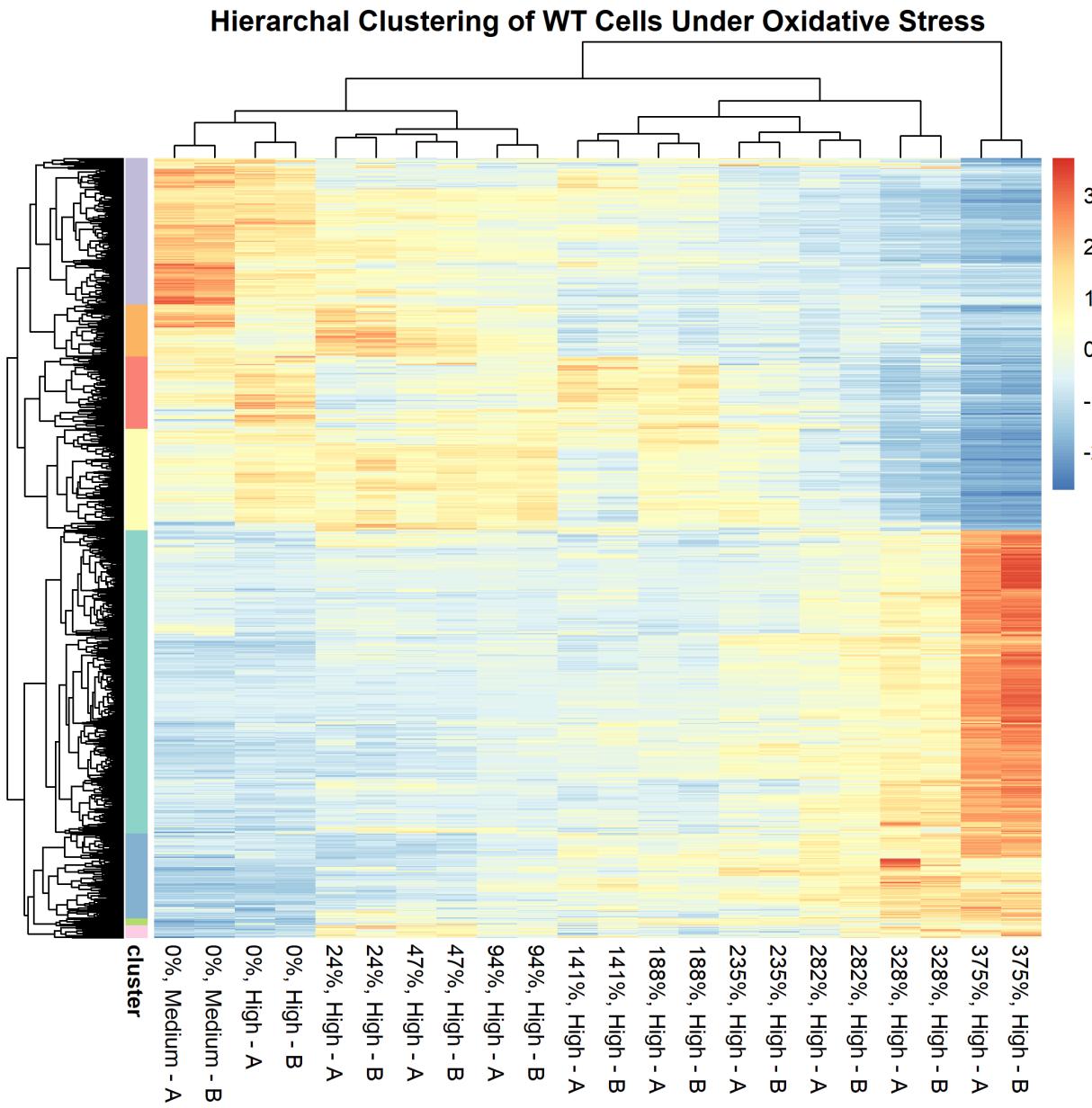


# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

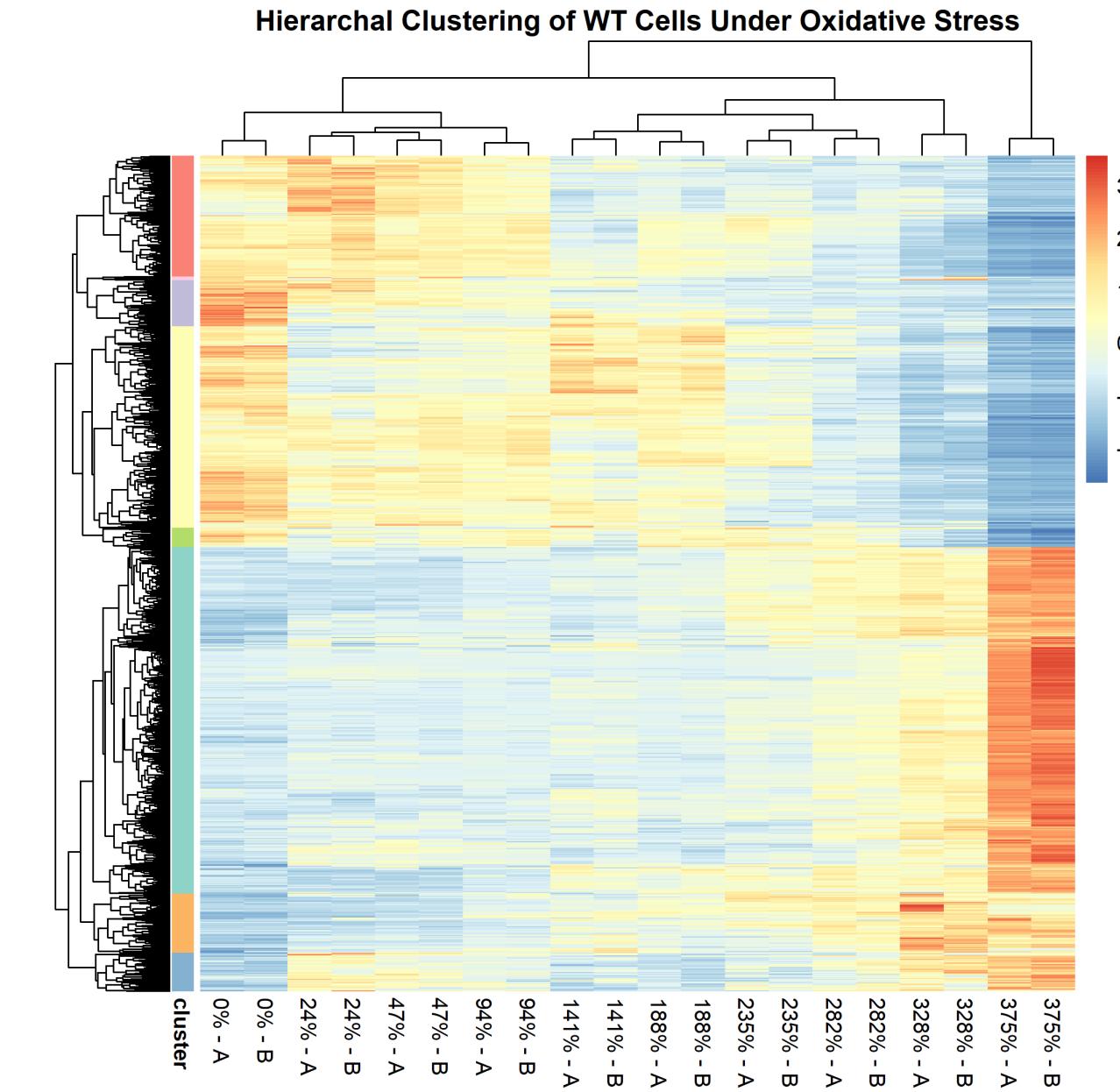
WT cyanobacteria gene expression adjustment in  
response of to oxidative stress



a) With medium light intensity sample



b) Without medium light intensity sample

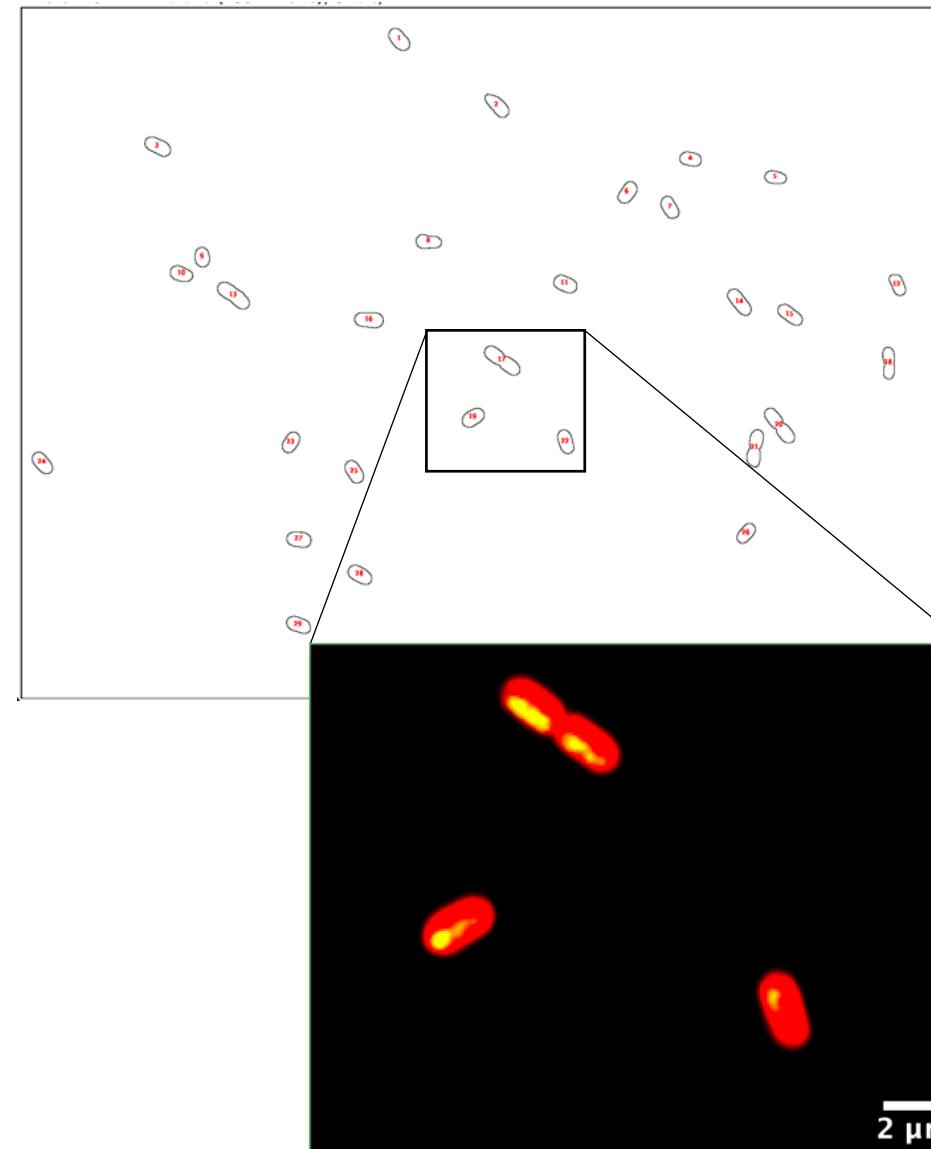


# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

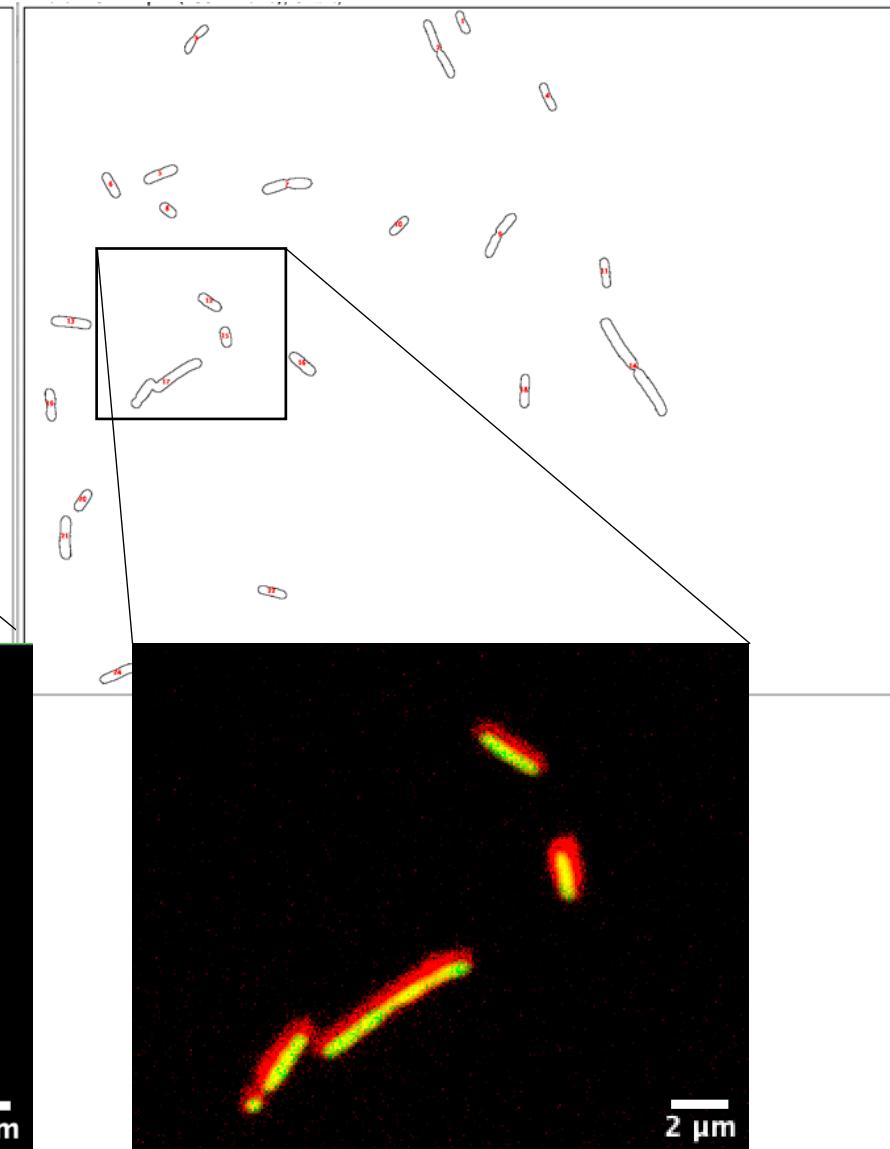


**WT cyanobacteria morphology adjustment in response of to oxidative stress**

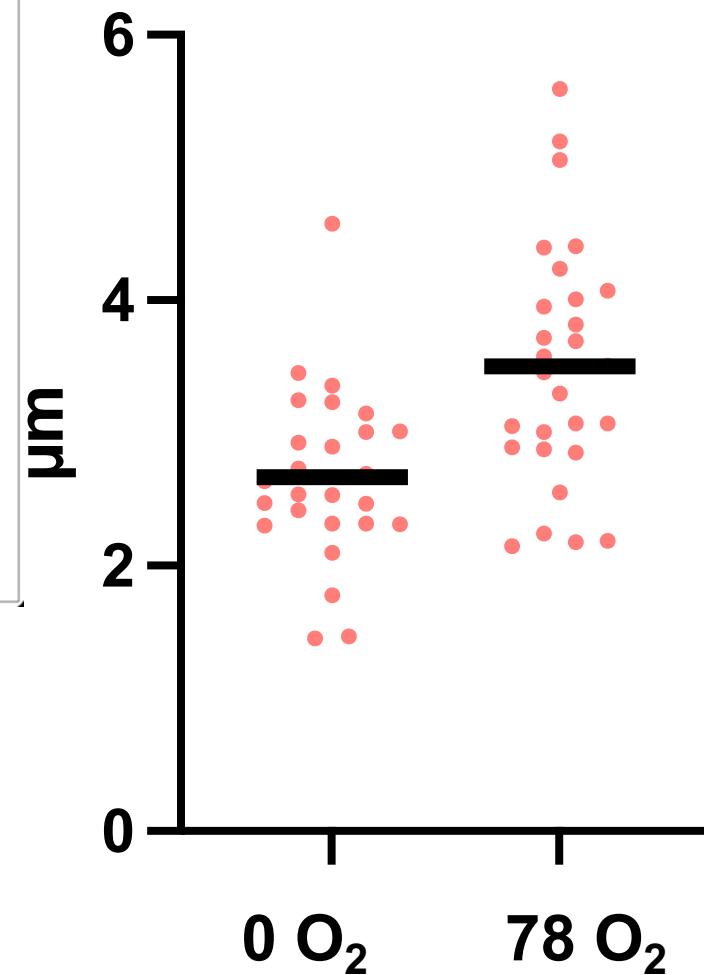
**WT 0 O<sub>2</sub> (low stress)**



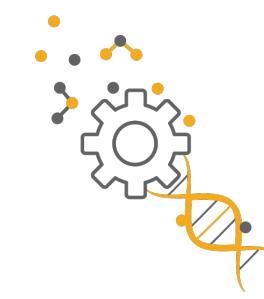
**WT 78 O<sub>2</sub> (high stress)**



**WT 0 vs. 78 O<sub>2</sub>**

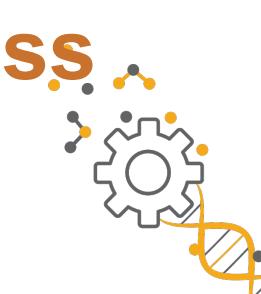


# List and description of stress conditions samples

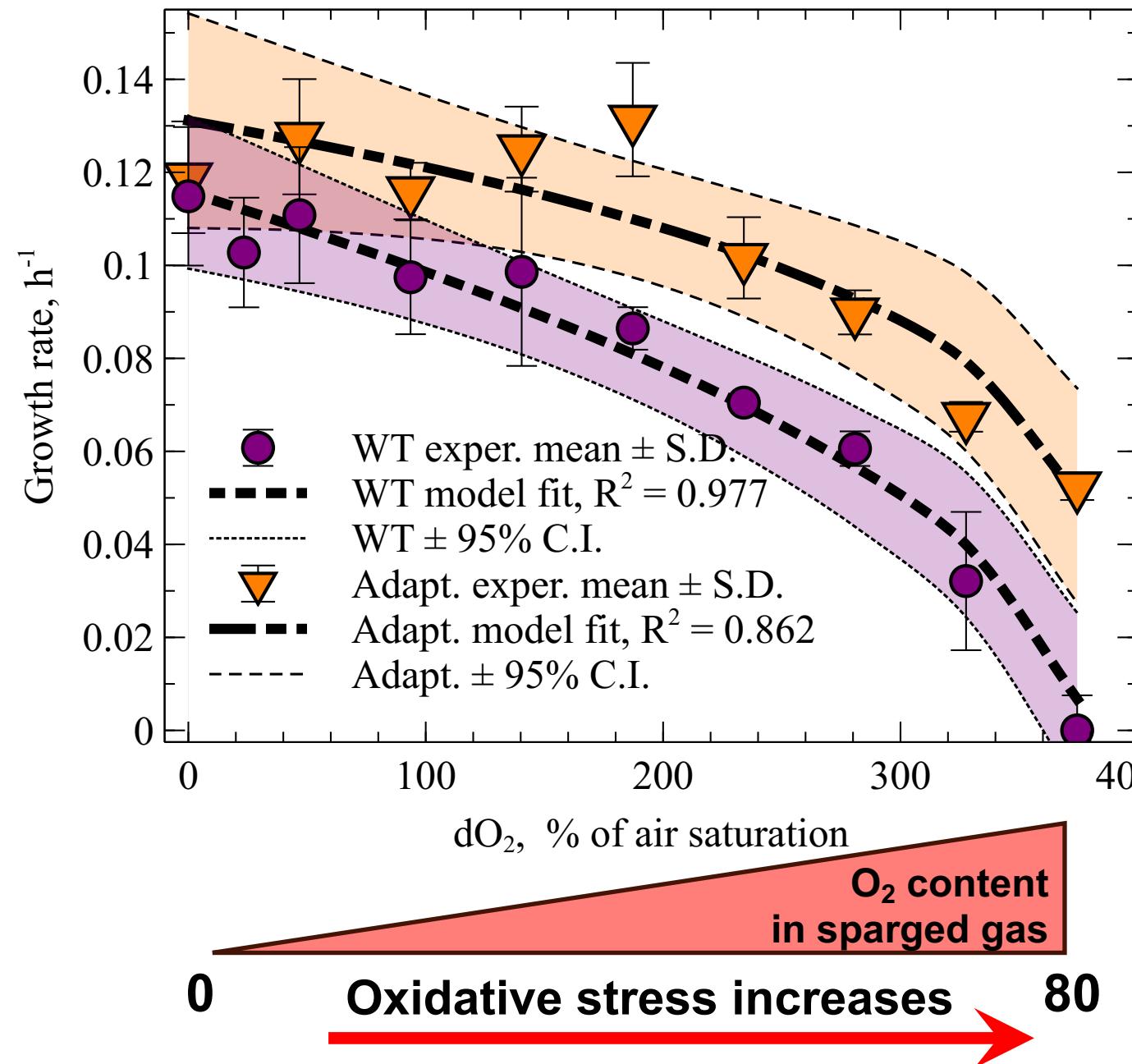


Sample		Gases sparged into PBR, L/min			O <sub>2</sub> in supplied gas, %	Dissolved O <sub>2</sub> from air saturation, %	Light	Steady state sample description	Note	
#	Name	CO <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>						
12.1	AD205.0A	0.04	2	0	0.0%	0.0	140:65	Adapted cells highlight and no O <sub>2</sub> stress (max growth rate of Adapted culture without stress)	Imposing oxidative stress on Adapted cyano by increasing O <sub>2</sub>	
12.2	AD205.0B	0.04	2	0	0.0%	0.0	140:65			
13.1	AD205.80A	0.04	0.4	1.6	78.4%	0.0	140:65	Adapted cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
13.2	AD205.80B	0.04	0.4	1.6	78.4%	0.0	140:65			
14.1	AD205.70A	0.04	0.6	1.4	68.6%	23.4	140:65	Adapted cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
14.2	AD205.70B	0.04	0.6	1.4	68.6%	23.4	140:65			
15.1	AD205.60A	0.04	0.8	1.2	58.8%	46.8	140:65	Adapted cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
15.2	AD205.60B	0.04	0.8	1.2	58.8%	46.8	140:65			
16.1	AD205.50A	0.04	1	1	49.0%	93.6	140:65	Adapted cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
16.2	AD205.50B	0.04	1	1	49.0%	93.6	140:65			
17.1	ADa.205.40A	0.04	1.2	0.8	39.2%	140	140:65	Adapted cells under high light and no O <sub>2</sub> stress (max growth rate reference)		
17.2	ADa.205.40B	0.04	1.2	0.8	39.2%	140	140:65			

# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

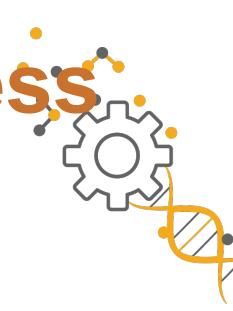


## WT vs Adapted cells growth rate phenotype adjustment in response to oxidative stress

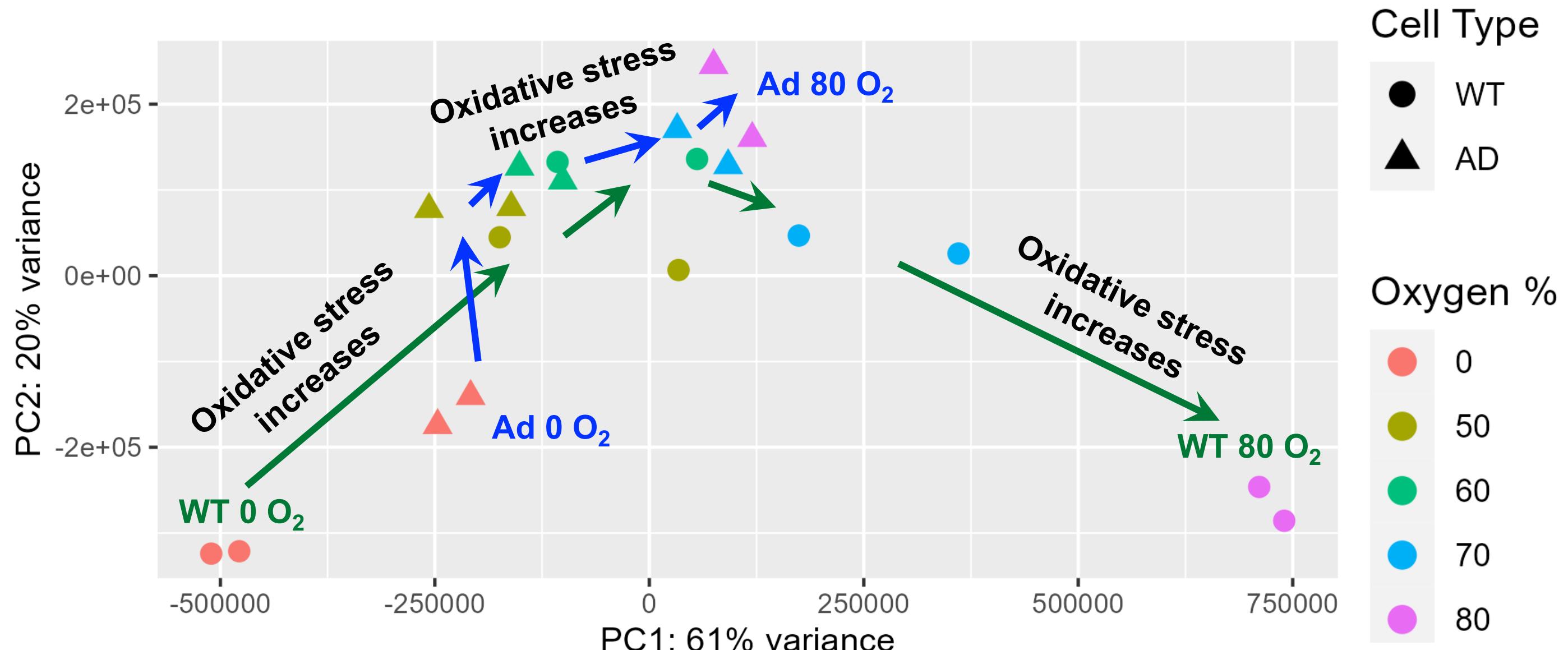


- growth rate of WT cells reduces along with increasing oxidative stress
- WT cells stop growing when  $\text{dO}_2$  reached  $\sim 375$  of air saturation
- culture was maintained at high oxidative stress to select adapted cells

# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions



WT vs. Adapted cells gene expression adjustment in response of to oxidative stress

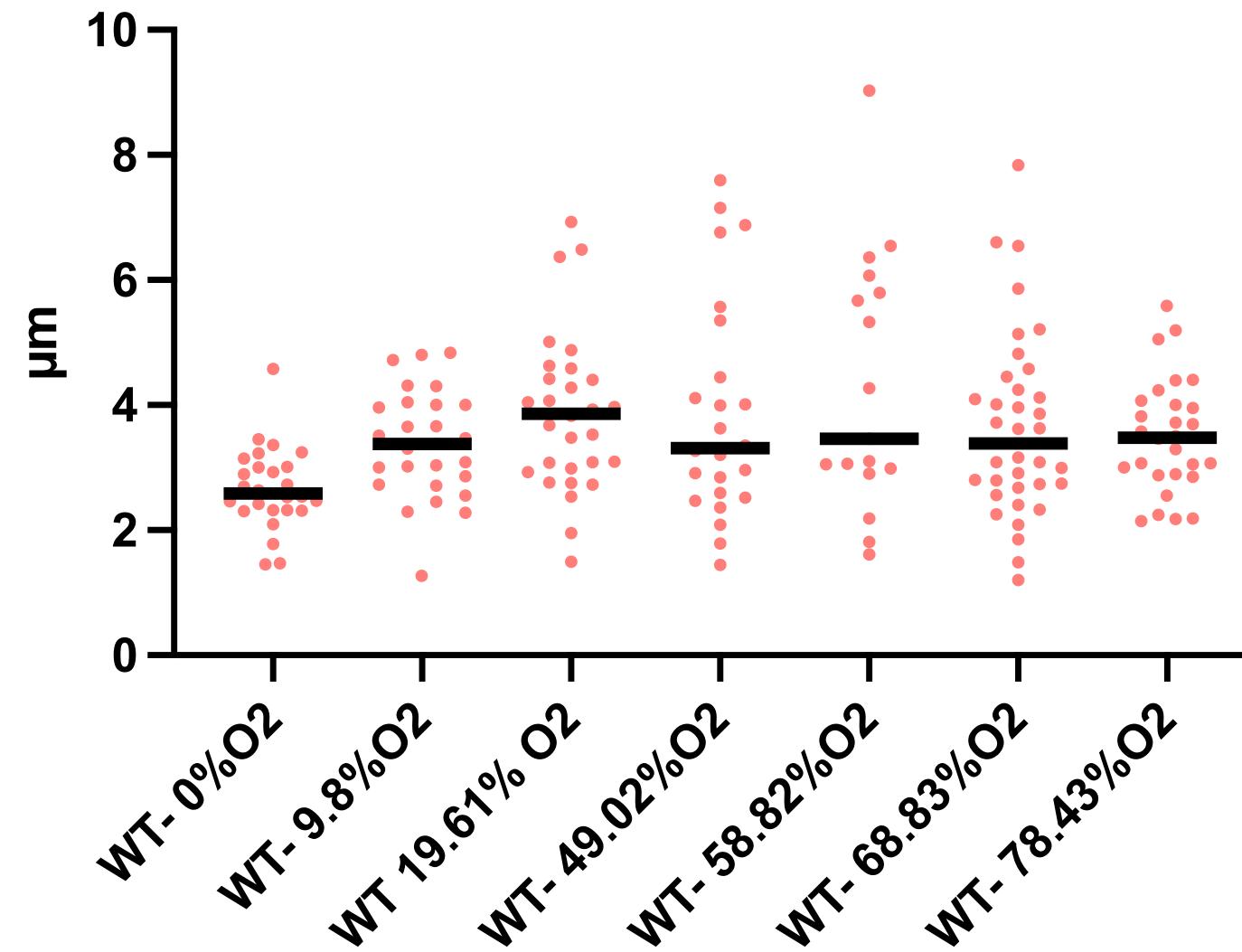


# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

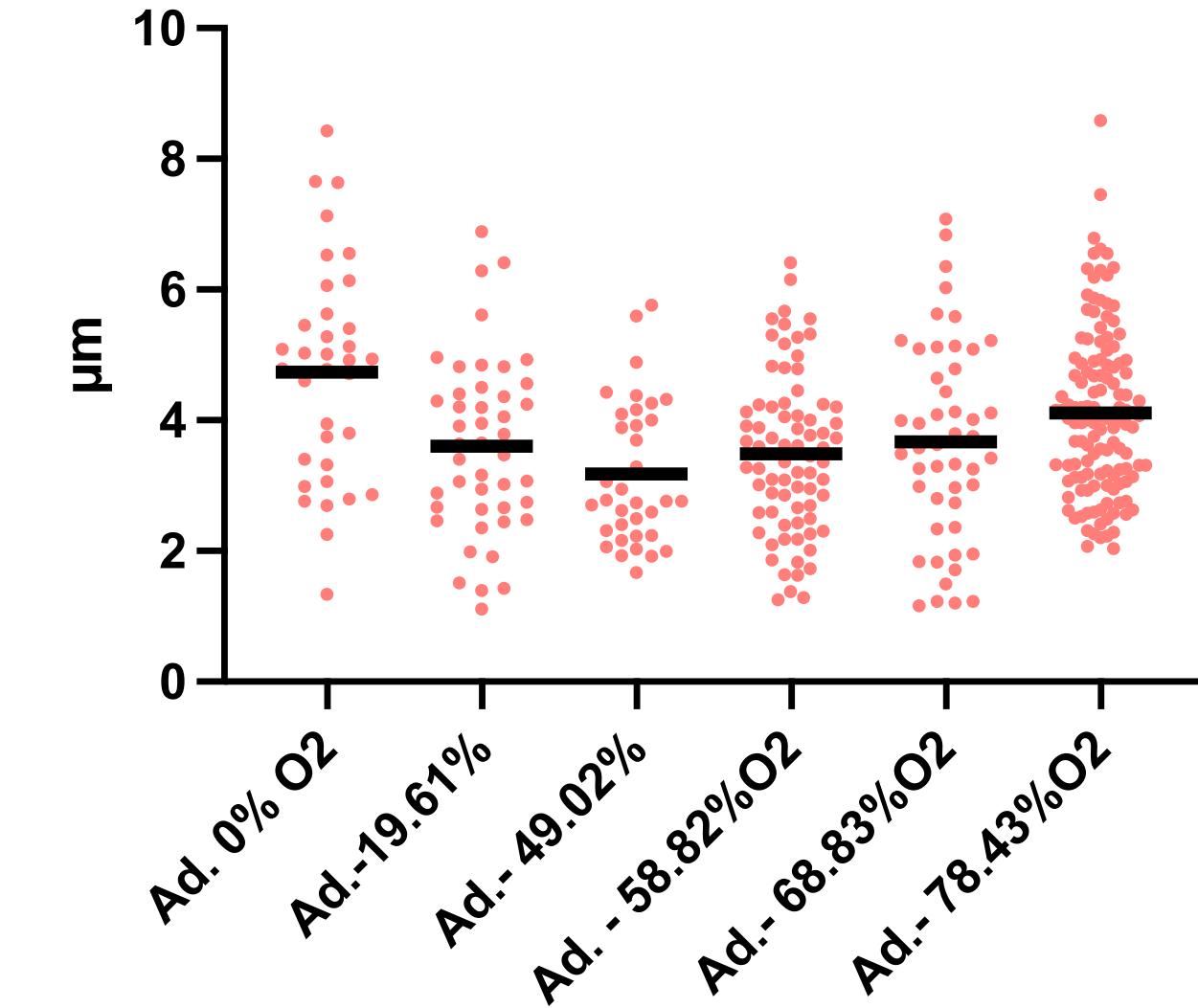


**WT vs Adapted cyanobacteria morphology adjustment in response of to oxidative stress**

**WT cell area**



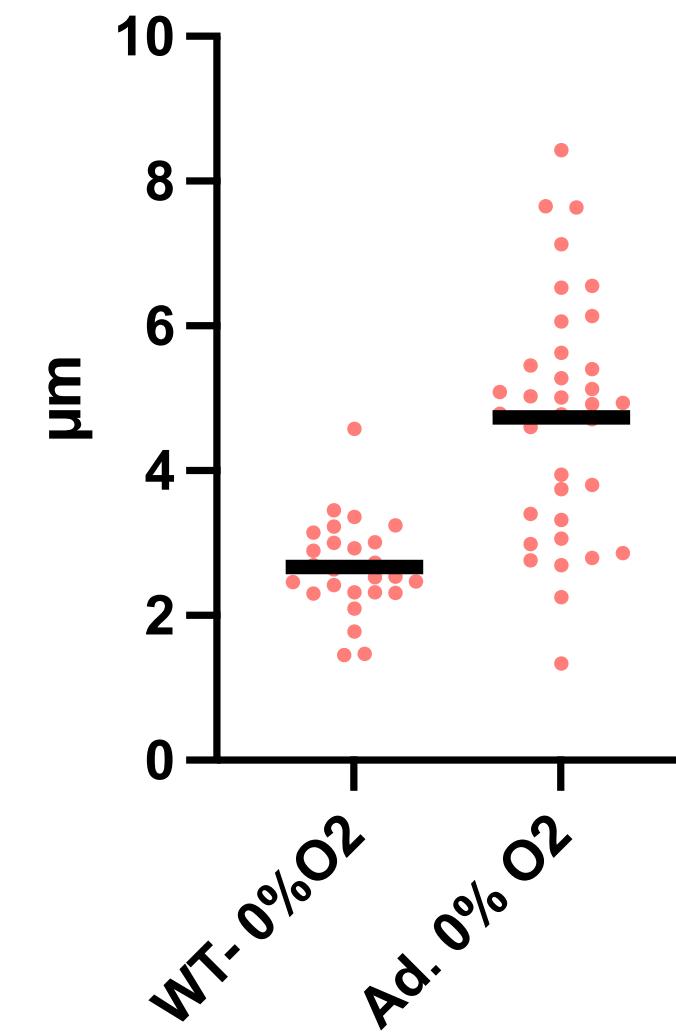
**Ad. cell area**



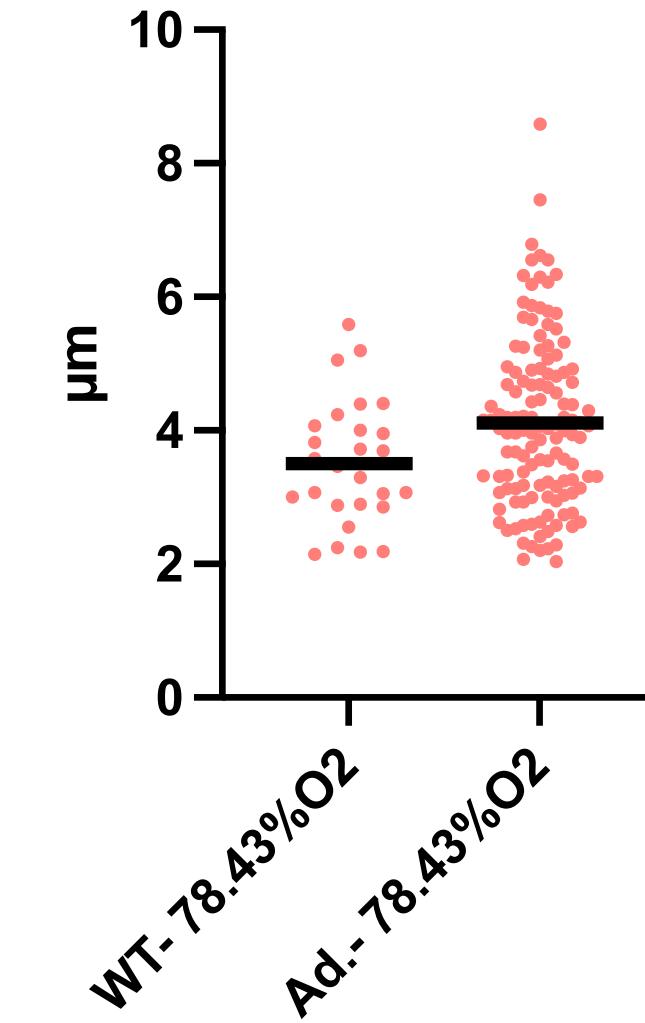
# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions

## WT vs Adapted cyanobacteria morphology

WT 0% vs Ad. 0% O<sub>2</sub> cell area



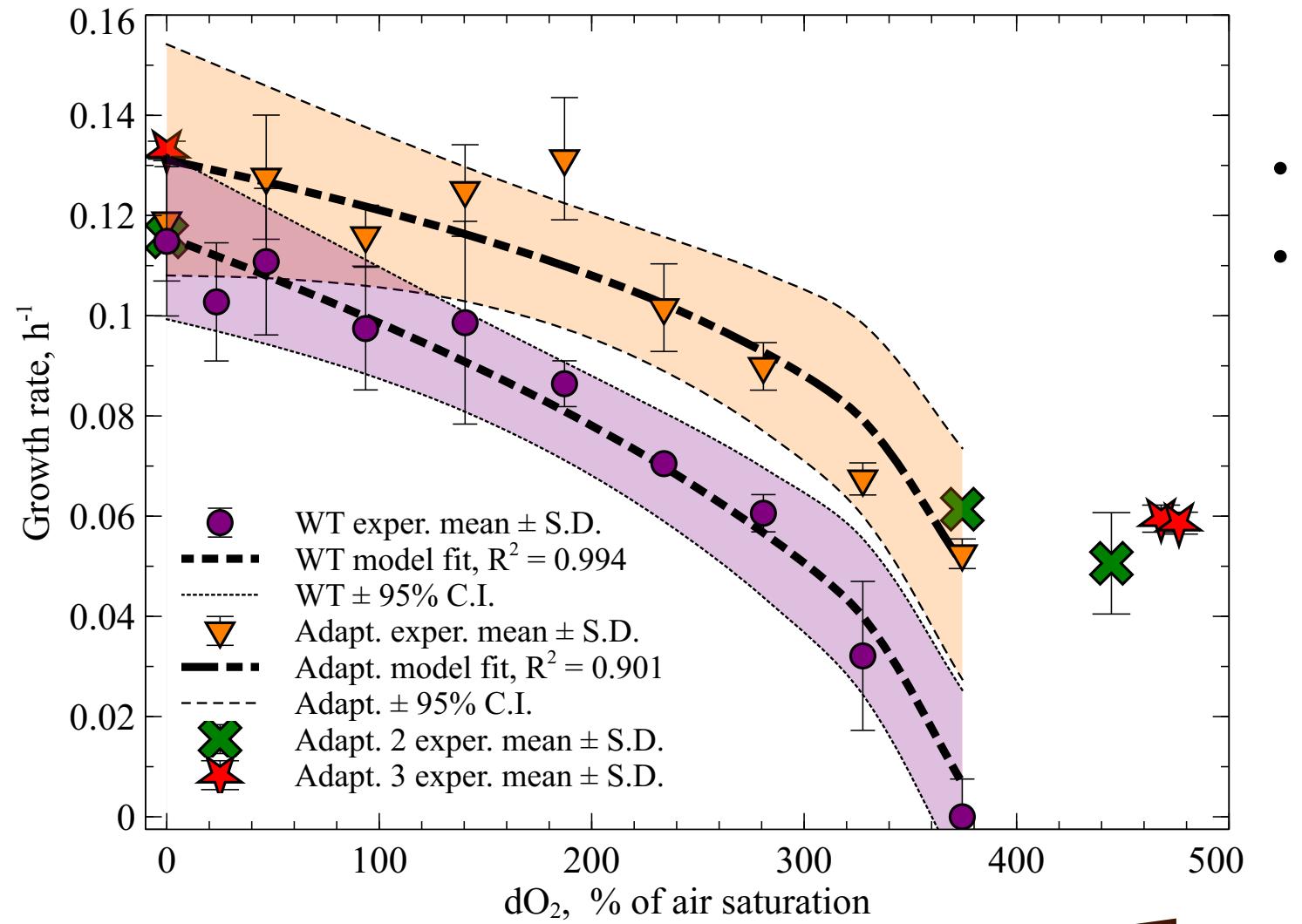
WT 78.43% O<sub>2</sub> vs Ad. 78.43% O<sub>2</sub>



# Phenotype 2: Enhanced cyanobacteria fitness under stress conditions



## Additional stress resistant Adapted cell lines



- Ad.2 cell line is able to grow at 93  $\text{O}_2$
- Ad.3 cell line is able to grow at 99.8  $\text{O}_2$

