Breker Lab

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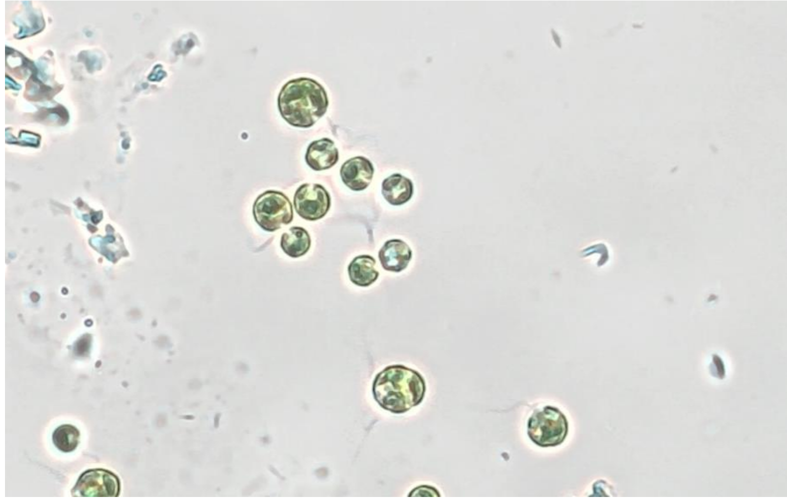
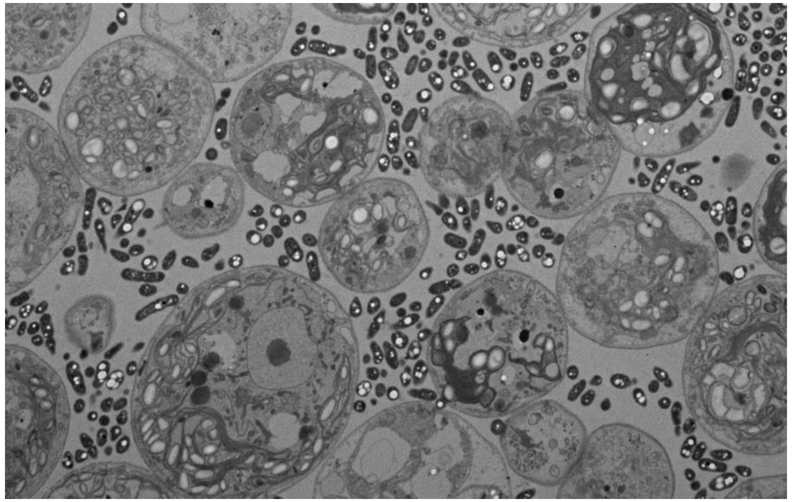
THE HEBREW UNIVERSITY OF JERUSALEM

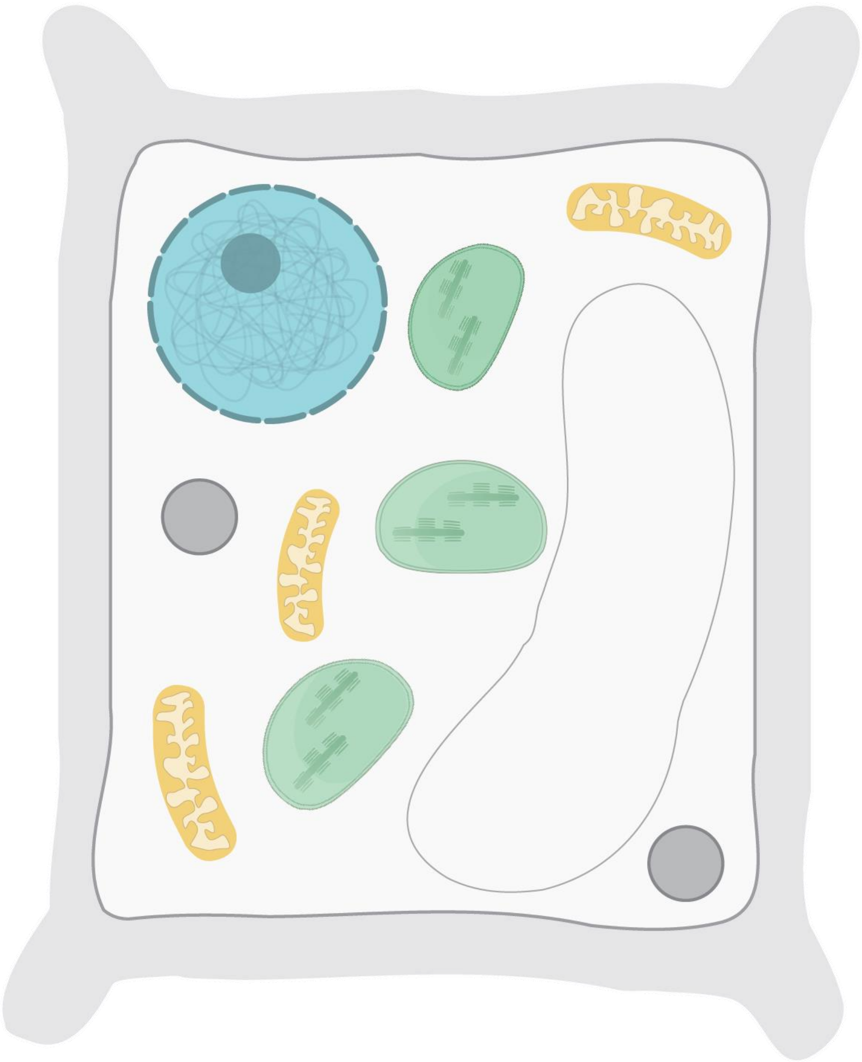
Systems genetics of microalgae for innovation in agriculture, medicine, and food Industries

Michal Breker-Dekel

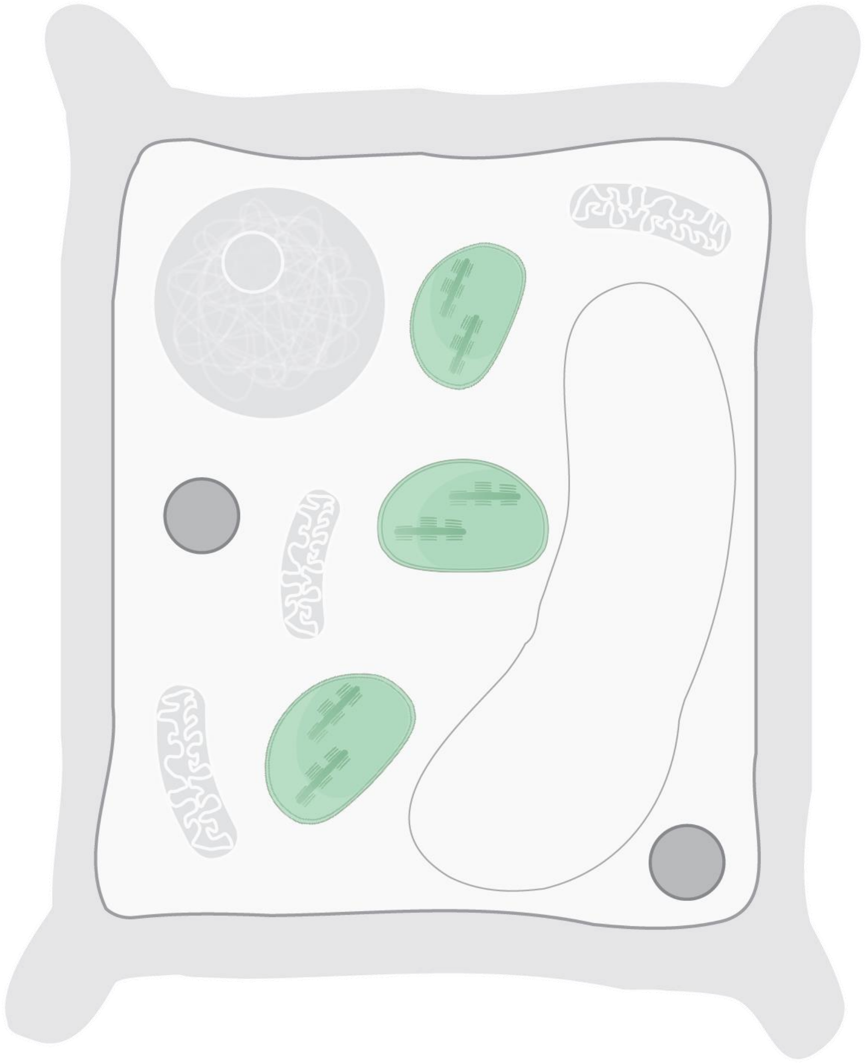




A look inside the plant cell: an elegant architecture of life



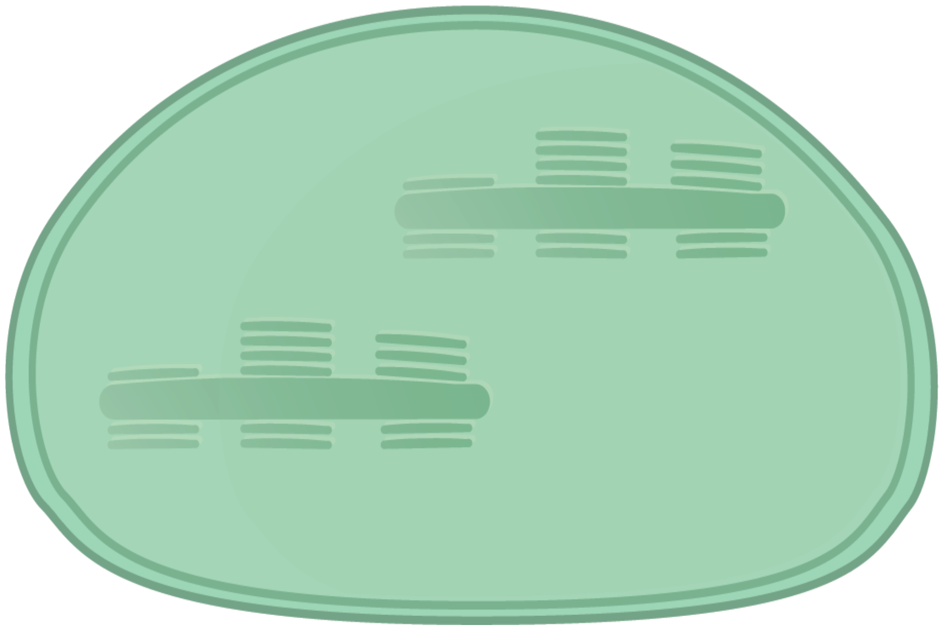
The chloroplast is the plant’s major energy producer

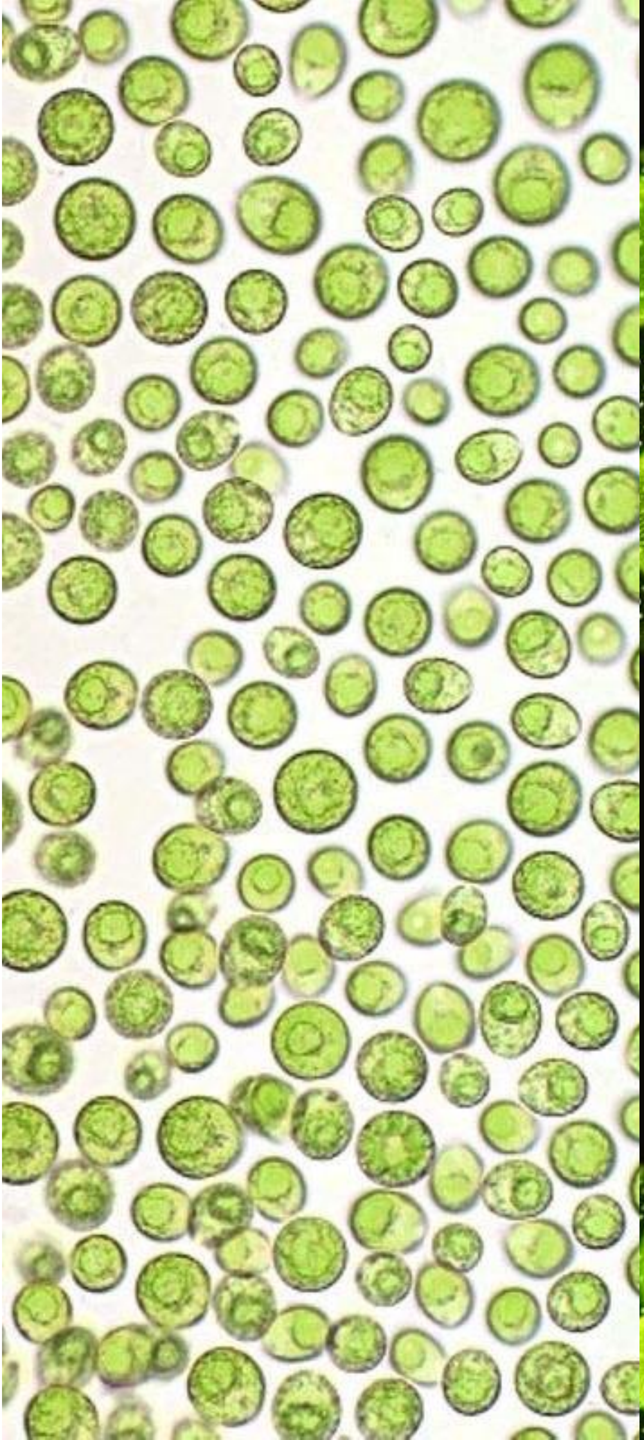
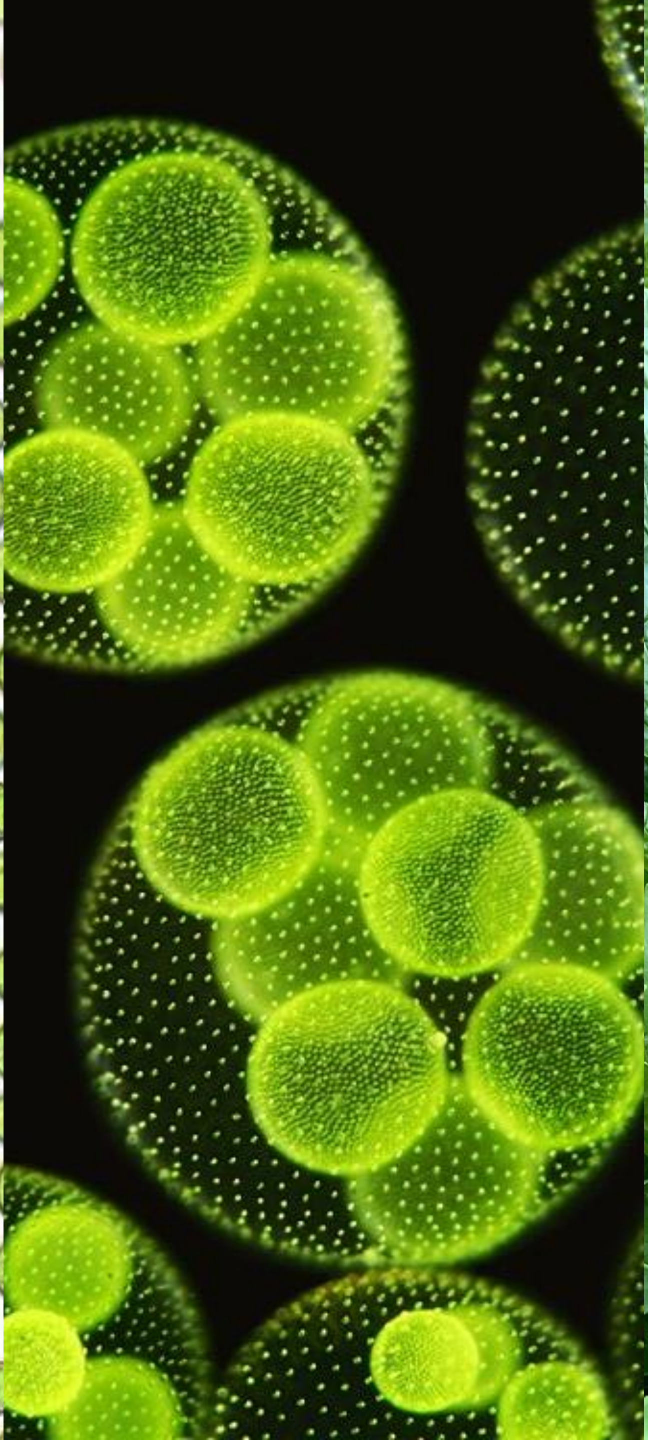


The chloroplast is the plant's major energy producer

- Photosynthesis
- Plant immune response
- Metabolic hub

Chloroplast



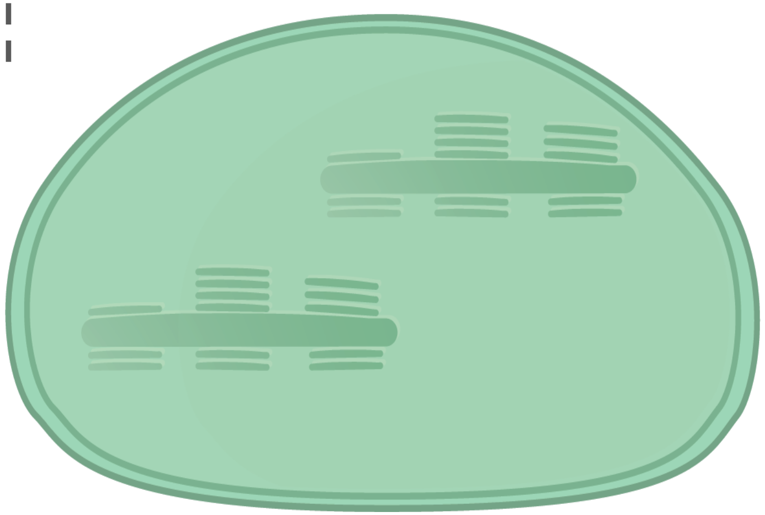



Organelle biology

Chloroplast contact sites

Protein quality control

cpUPR = chloroplast unfolded protein response



Plant cell biology

Essential pathways

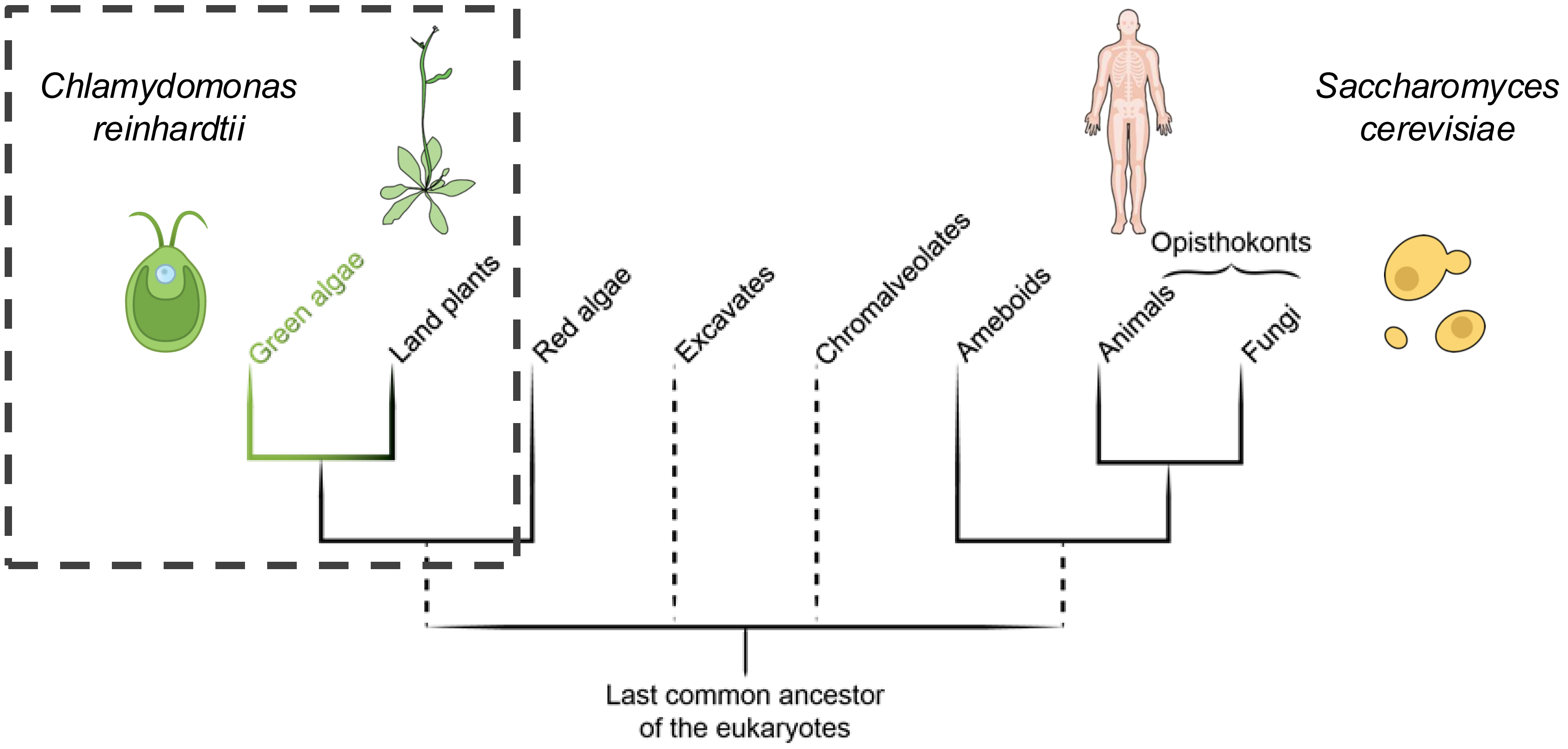
Organelle biogenesis

Targeting and translocation pathways

Translocon clogging

Tic/Toc modulation

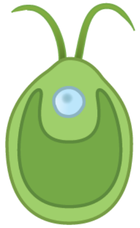
Simple model system to study plant cell biology



Tree adapted from: Rogozin IB *et al*, Genome Biol Evo 2009

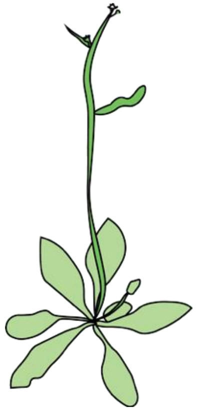
Simple model system to study plant cell biology

Chlamydomonas reinhardtii



- **Unicellular**
- Short generation time
- **Haploid** and **single copy** genes

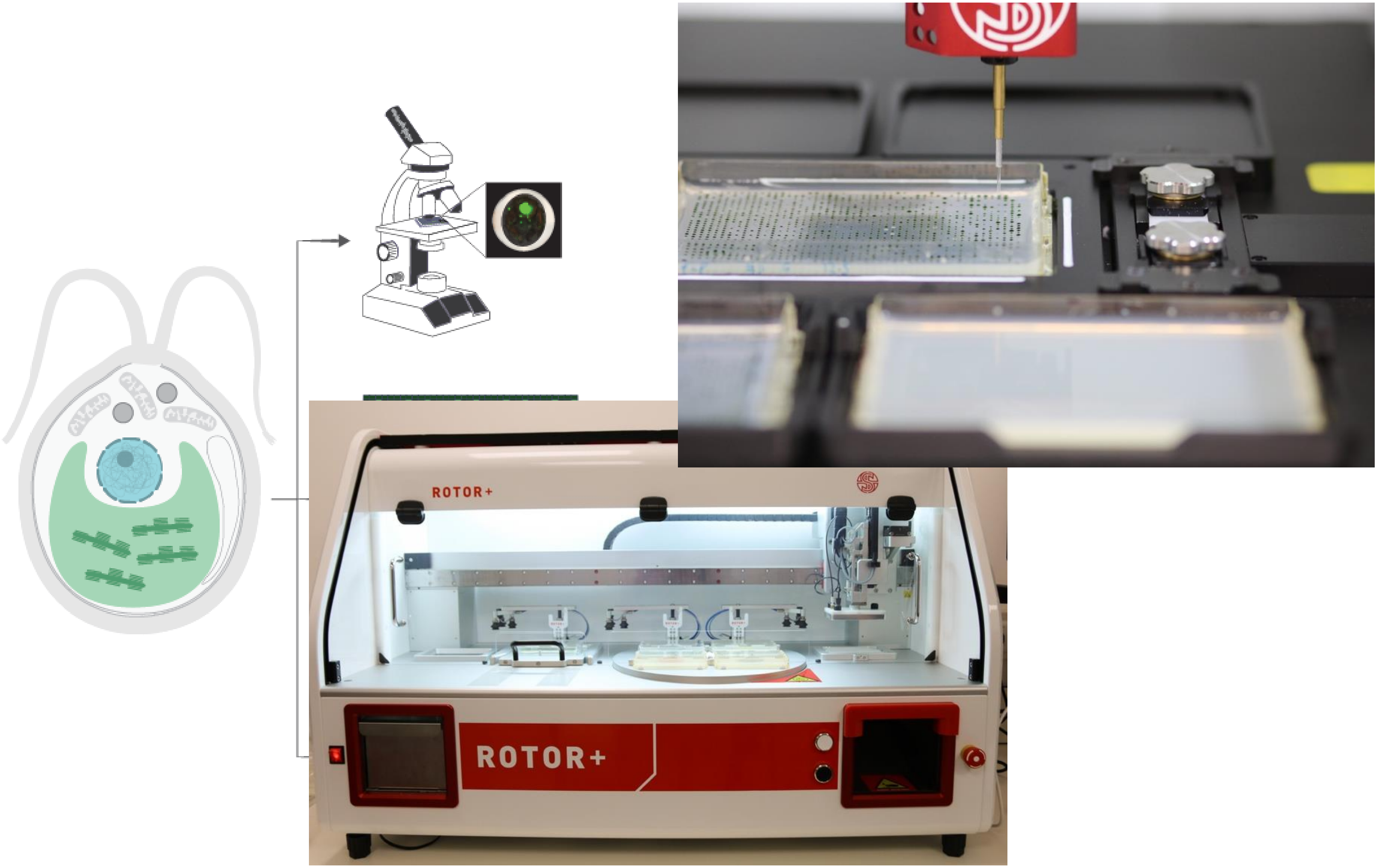
Land plant models

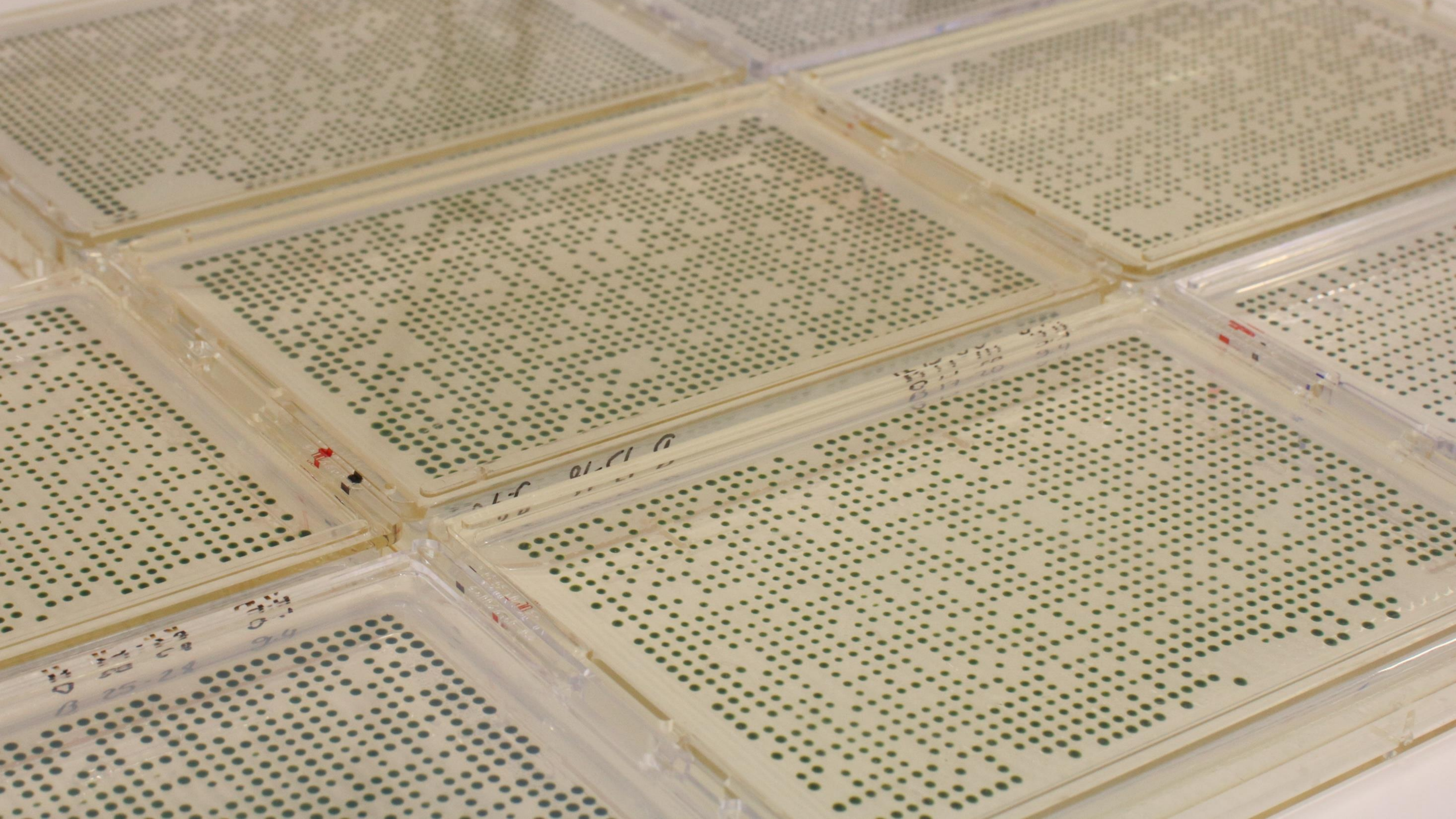


- **Multicellular**
- Long generation time
- **Polyploids**

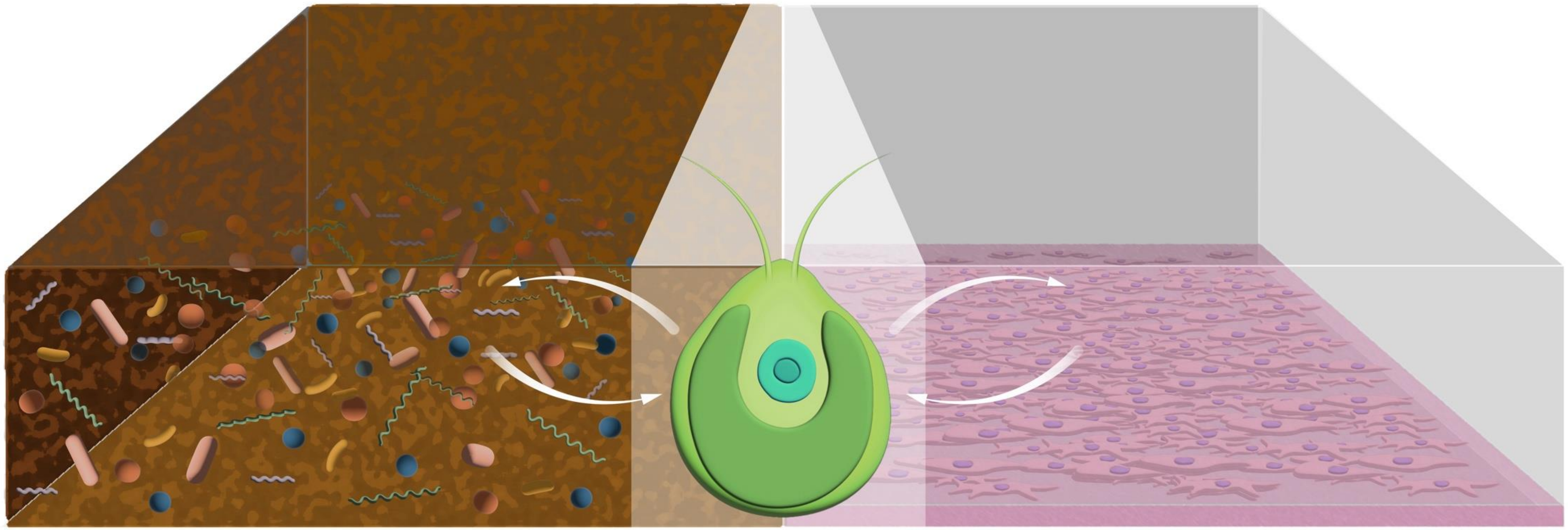
Tree adapted from: Rogozin IB *et al*, Genome Biol Evo 2009

Automated-based procedures enable to scale up throughput





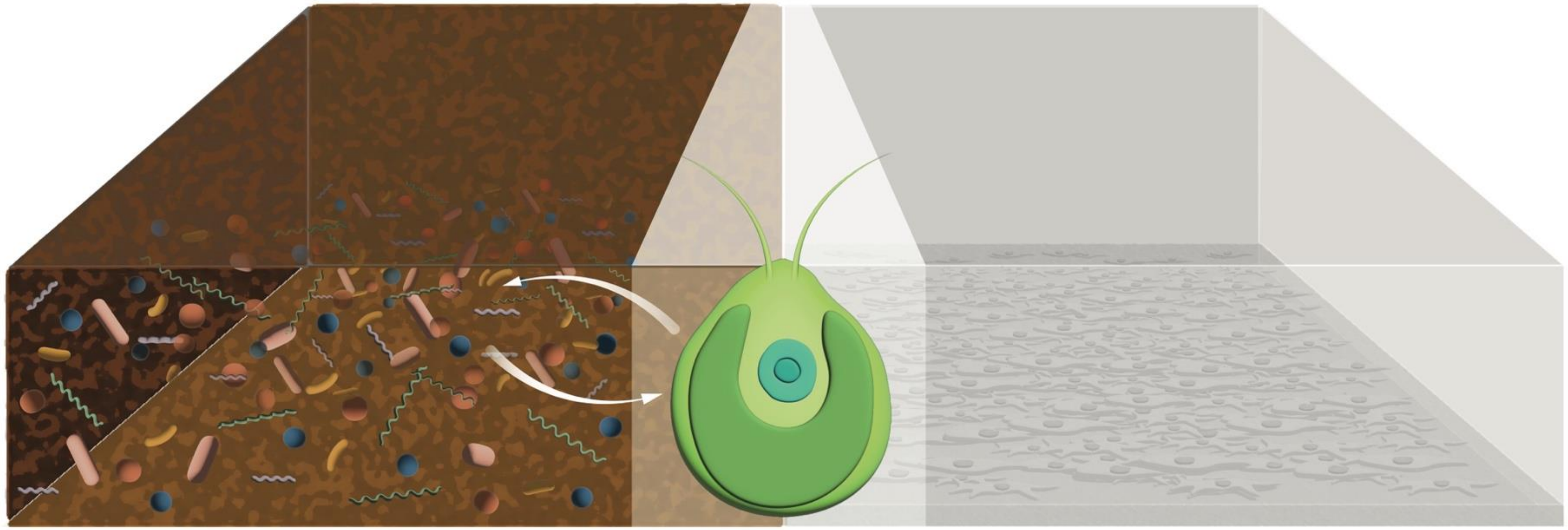
Beyond the cell: the social life of microalgae



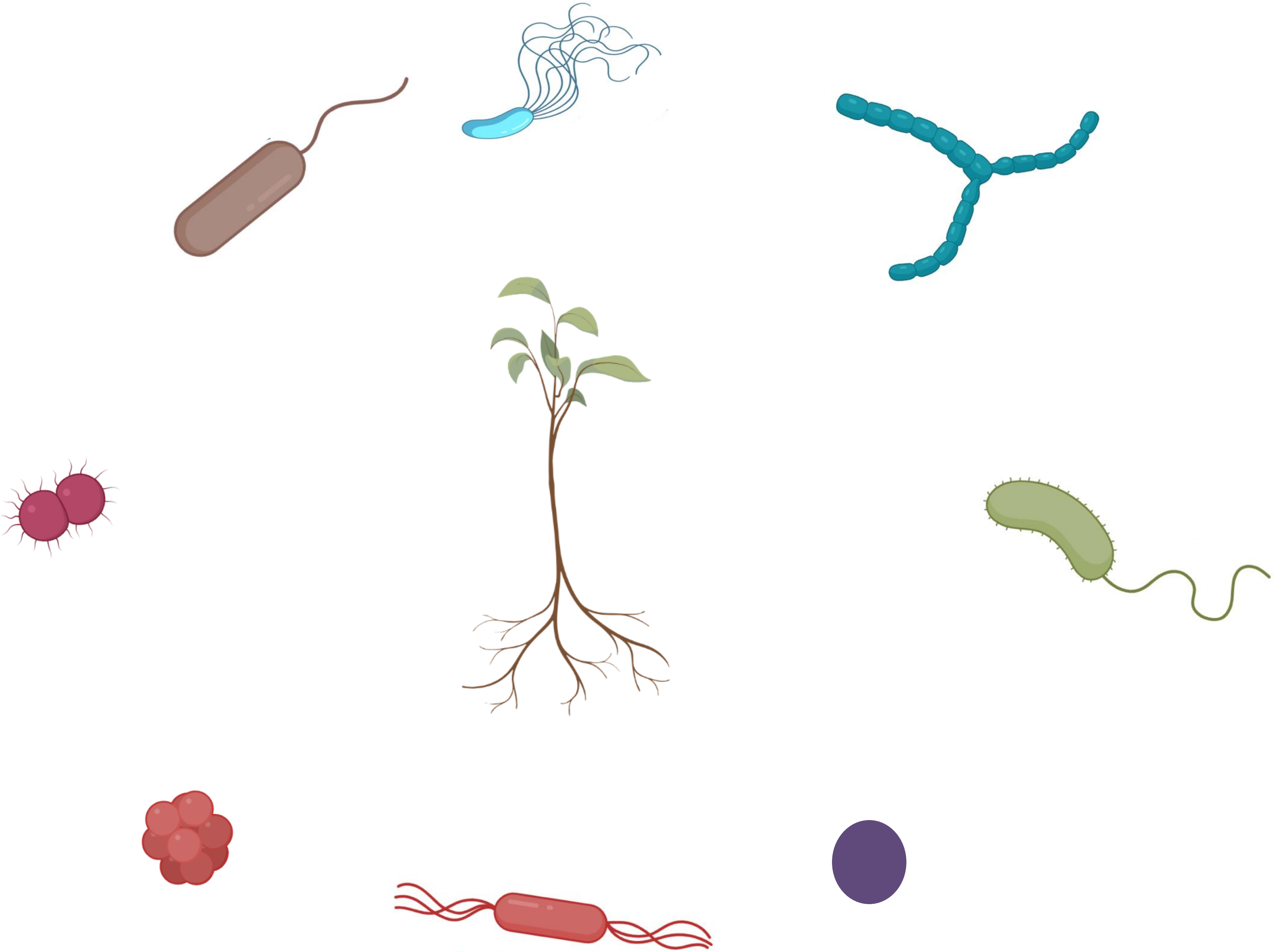
Natural soil microbiome

Synthetic ecosystem with animal culture

Deep evolutionary conservation of bacterial antagonism towards plants

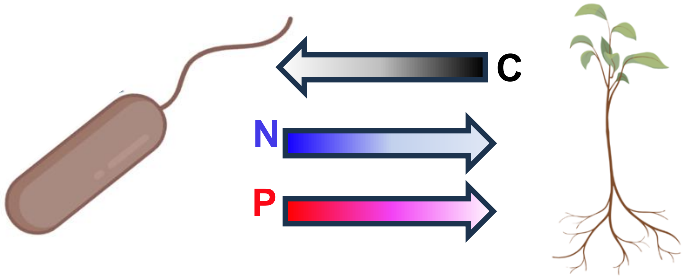


Dr. Omri Finkel Tzila Vikinyansky Elad Meilin

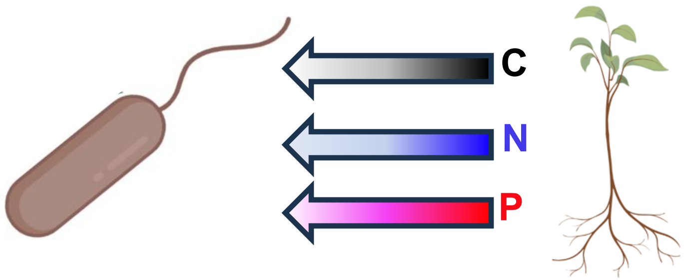


Nutritional symbioses is a common evolutionary strategy

Mutualist



Pathogen

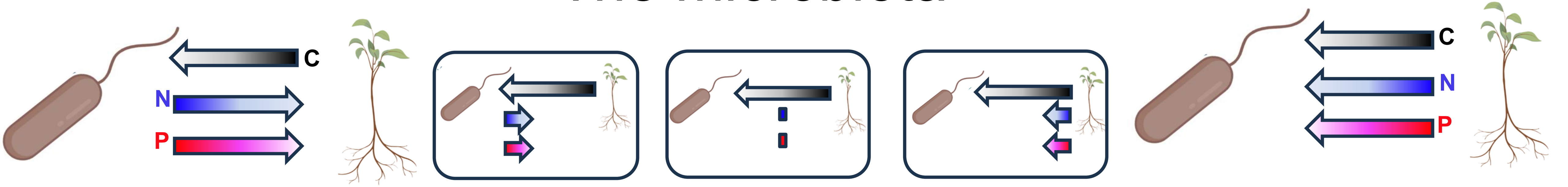


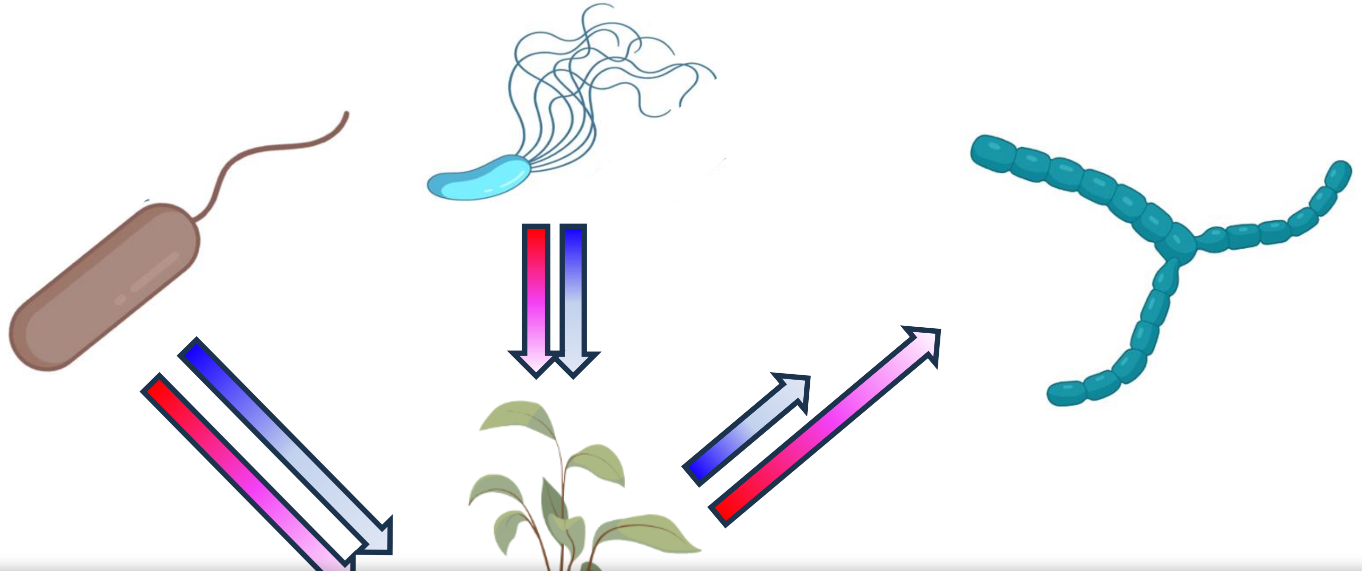
The soil microbiota ranges within broad landscape of symbioses modes

Mutualist

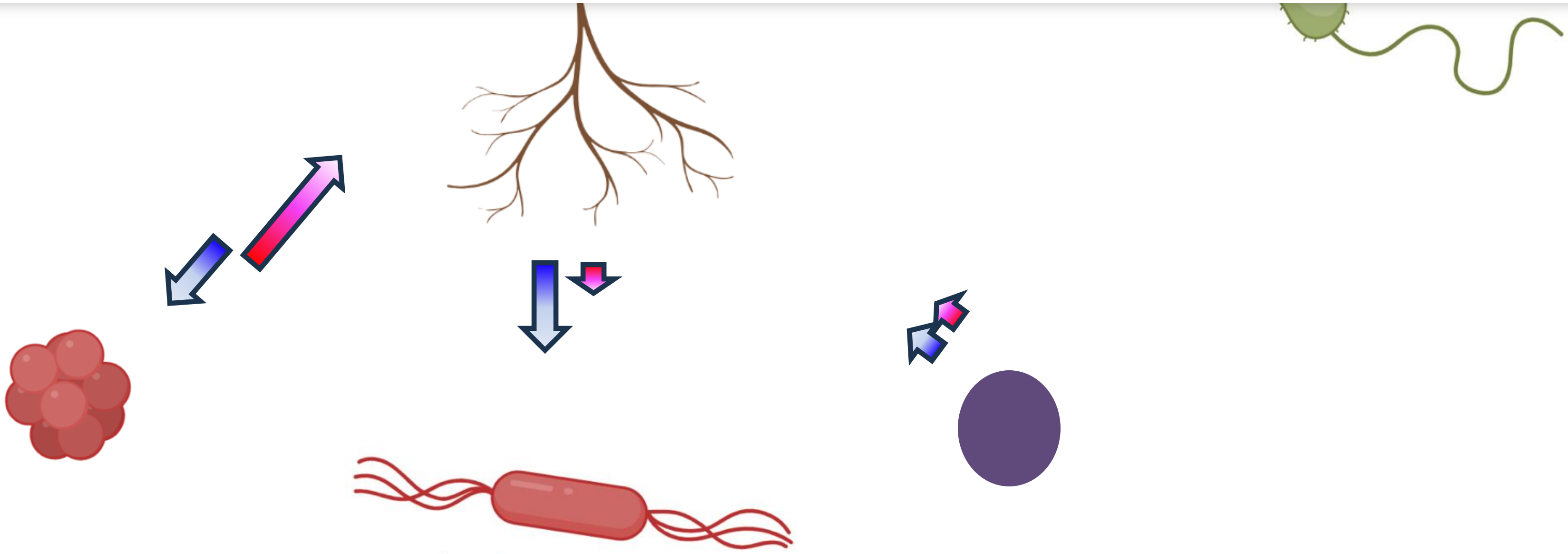
Pathogen

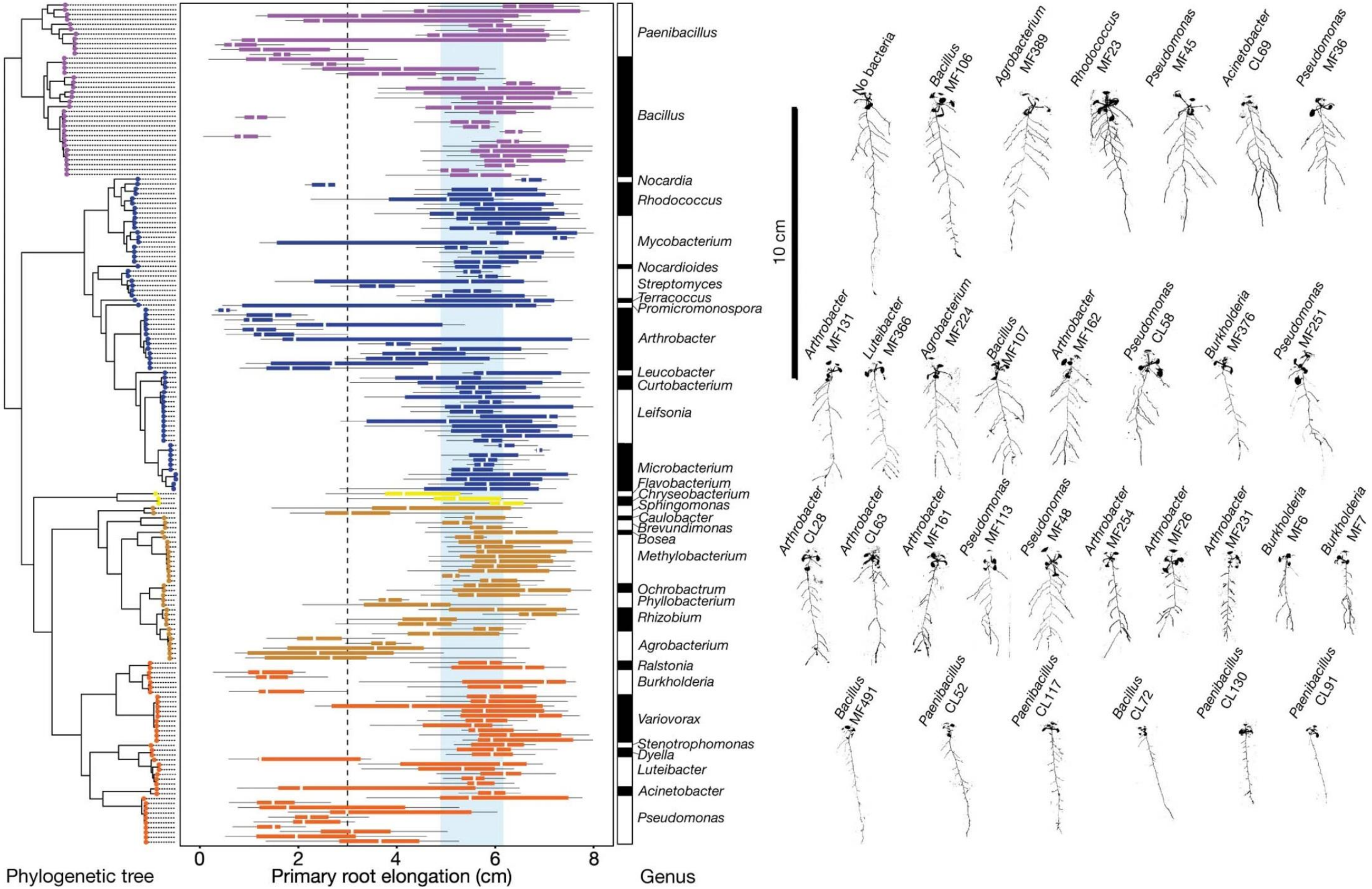
The microbiota





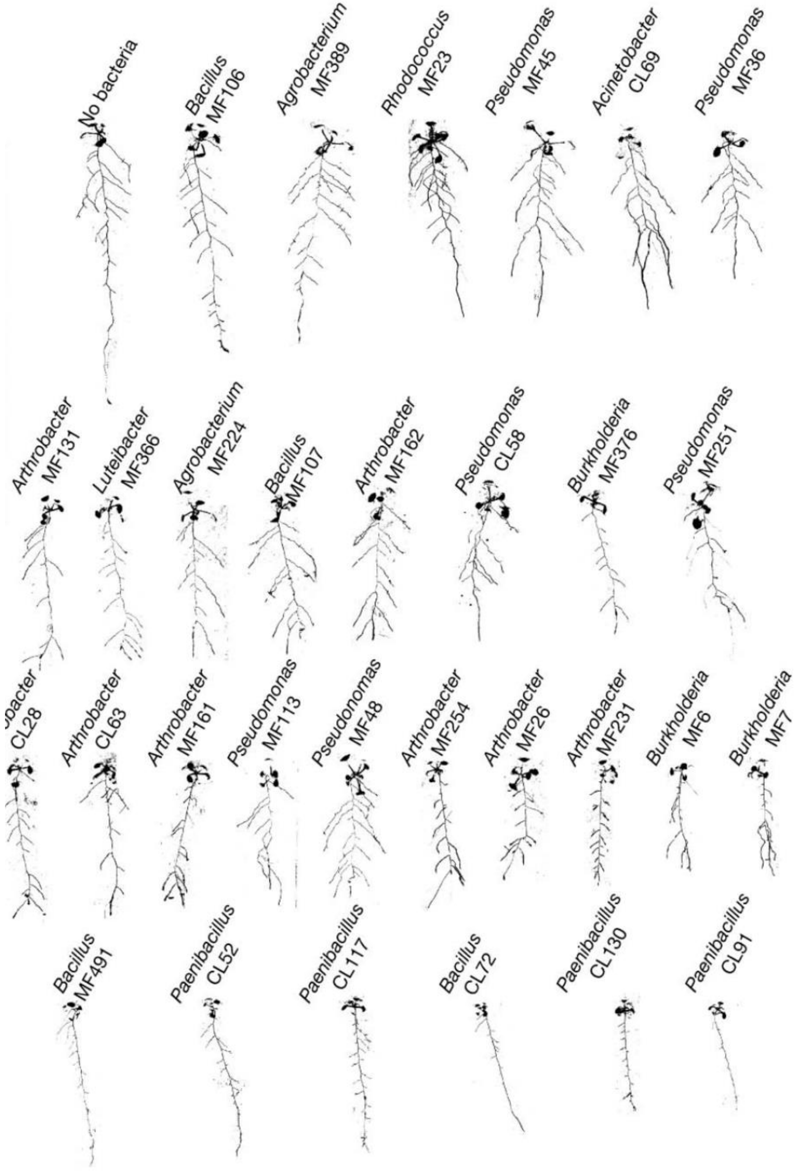
The goal would be to shift the balance towards mutualism for the benefit of the plant





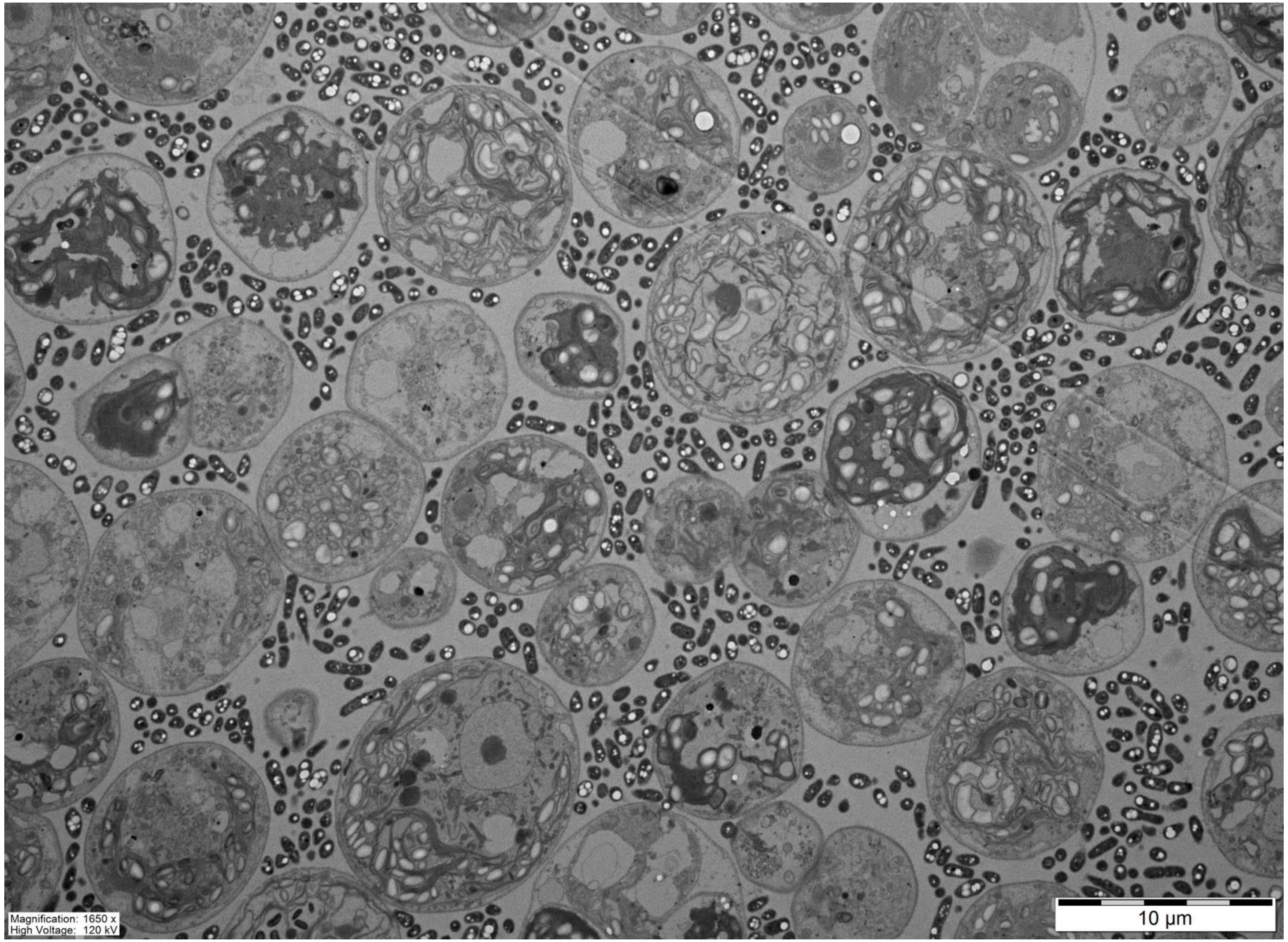
Finkel et al, 2020

- How much of the soil microbiota is antagonistic to plants?
- How can we rapidly identify antagonists?
- Is antagonism conserved in evolution?
- What mechanisms are employed?

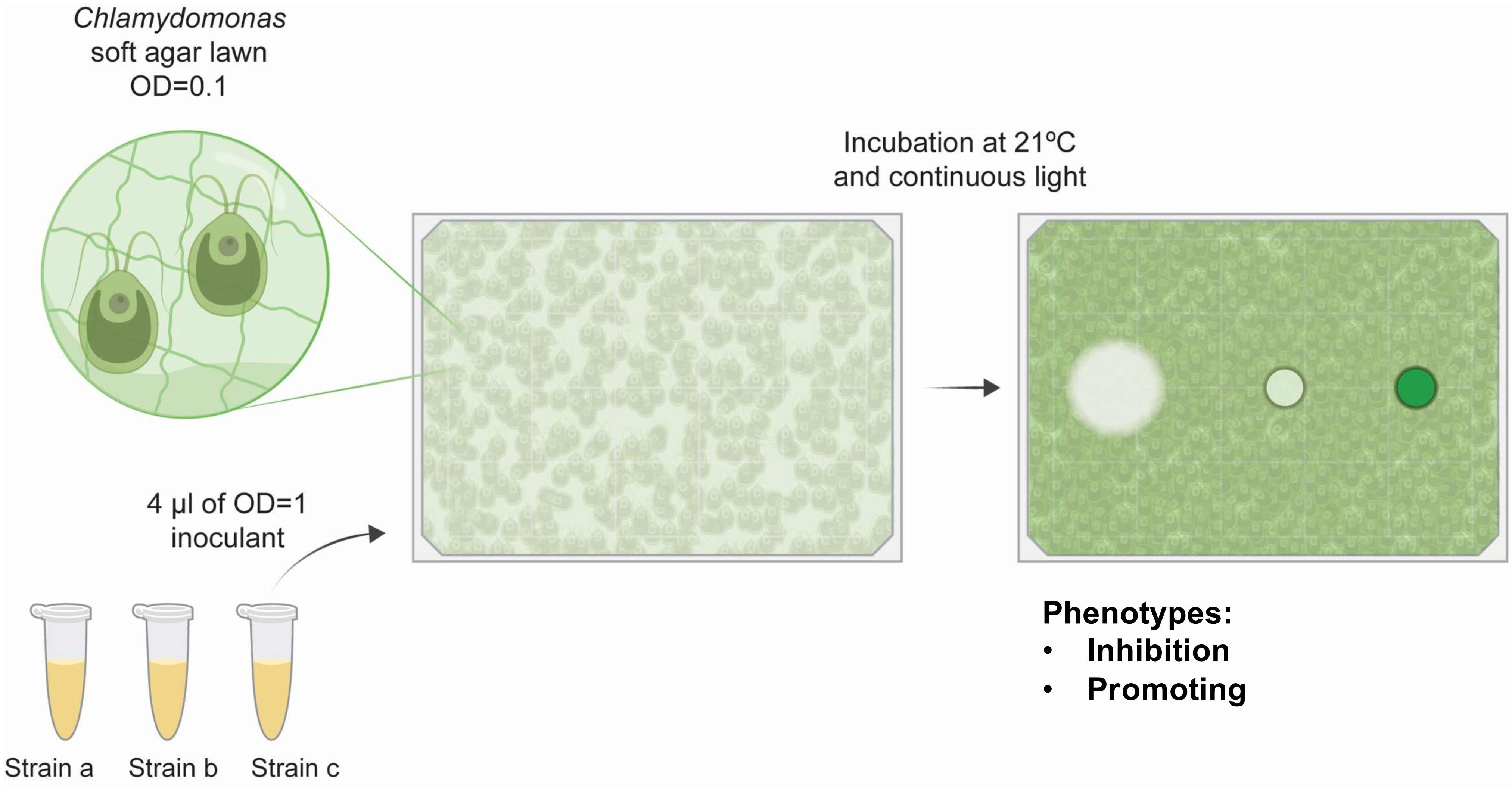


Finkel et al, 2020

Can we detect conservation in bacterial antagonism towards algae and plants?

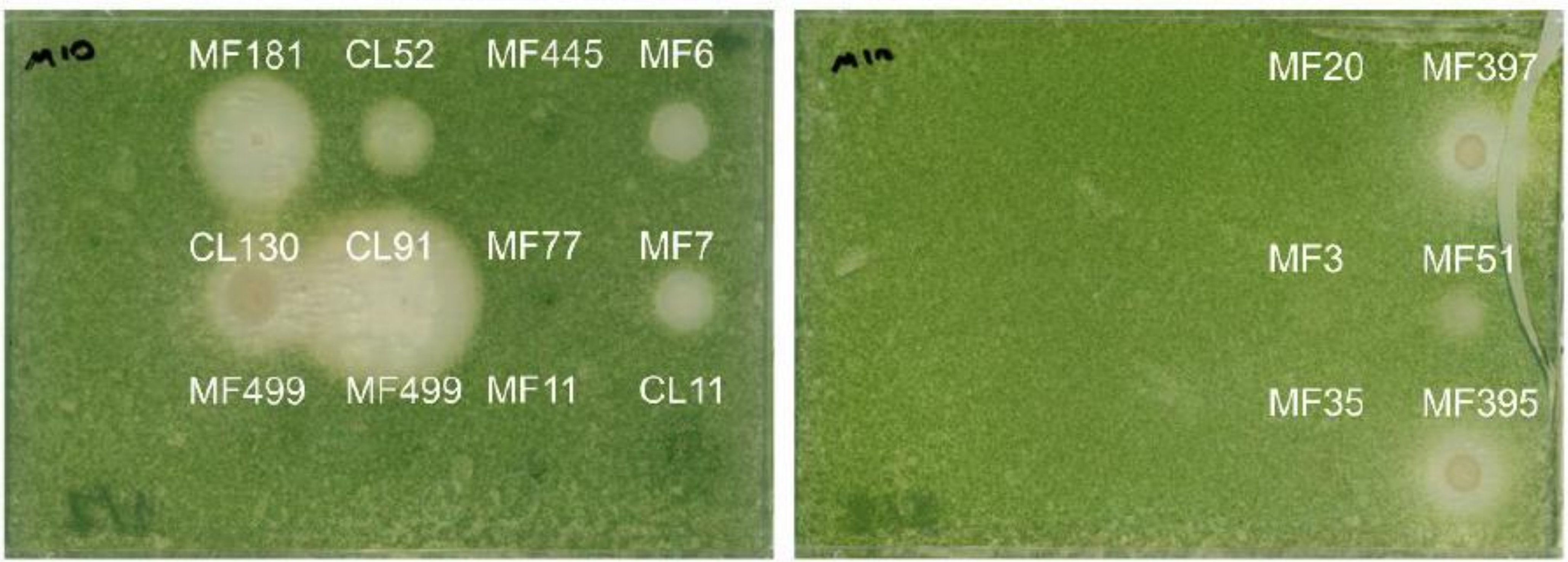


Establishing a robust method to screen for bacteria-*Chlamydomonas* interactions

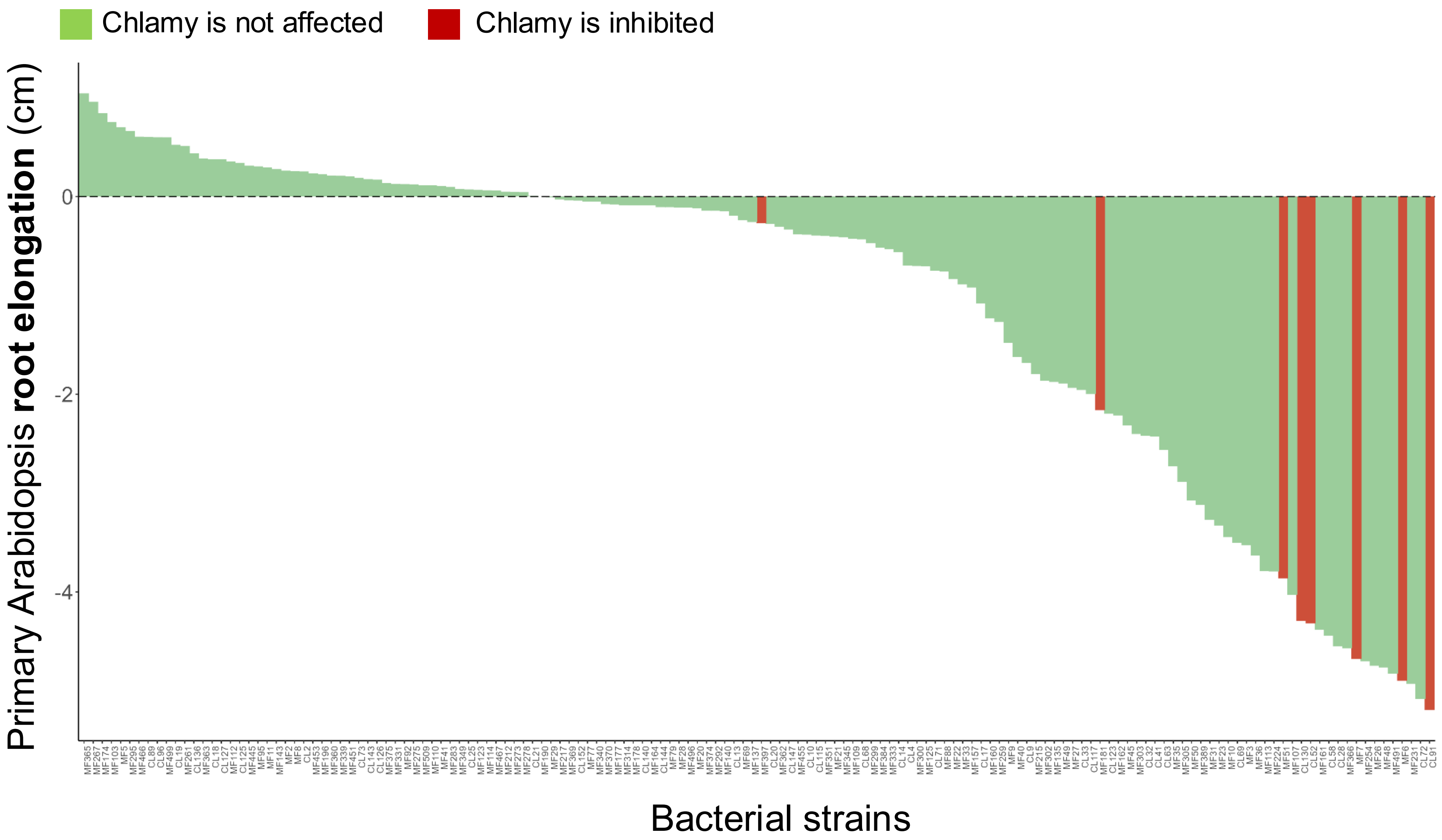


Phenotypes:
- **Inhibition**
- **Promoting**

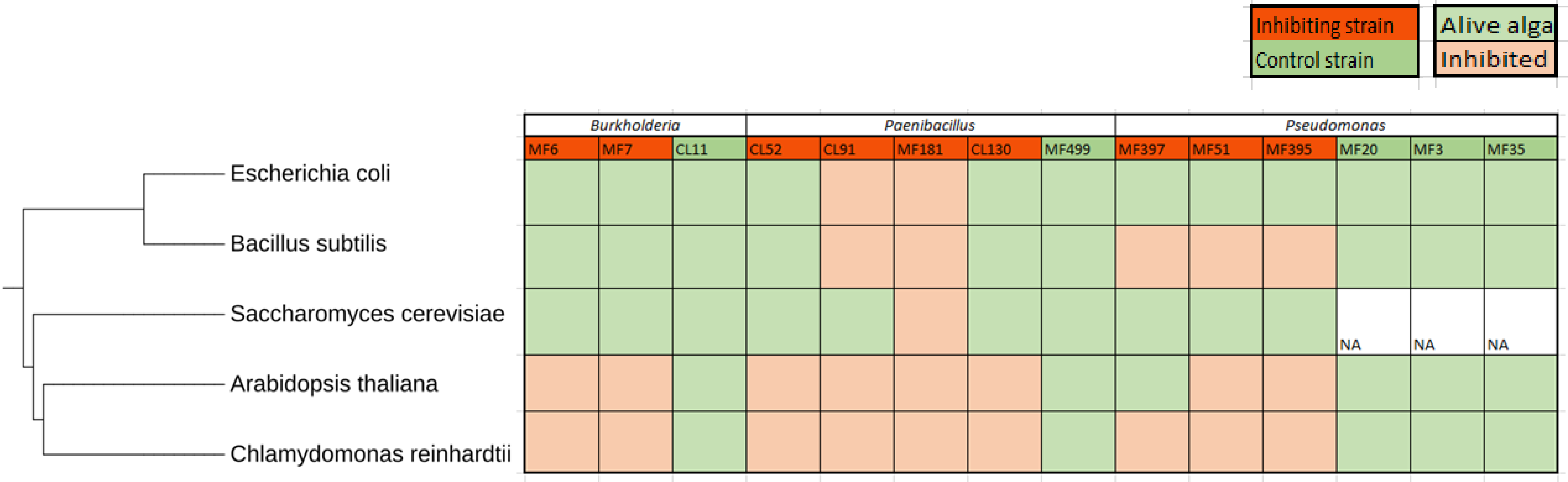
<u>Nine</u> bacterial species form halos out of the 173 tested



All *Chlamydomonas* inhibitors are also plant inhibitors

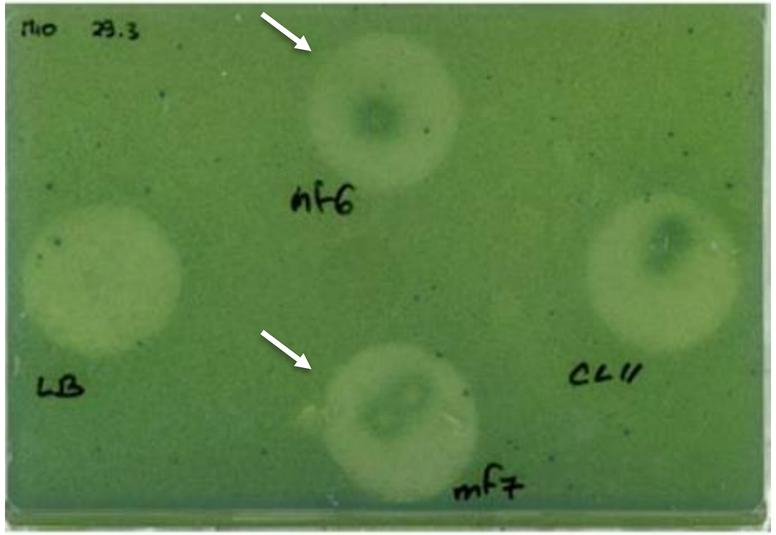


Burkholderia and *Paenibacillus* are green lineage specific

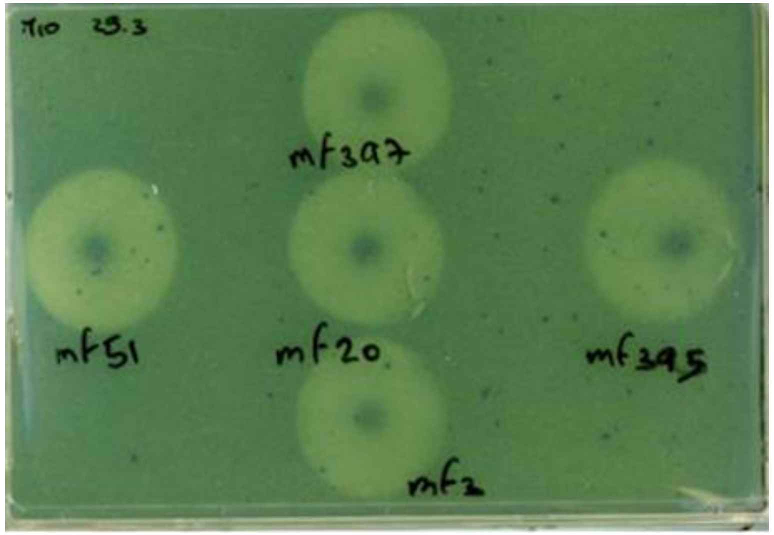


Filter-based assay was performed to dissect contact dependency

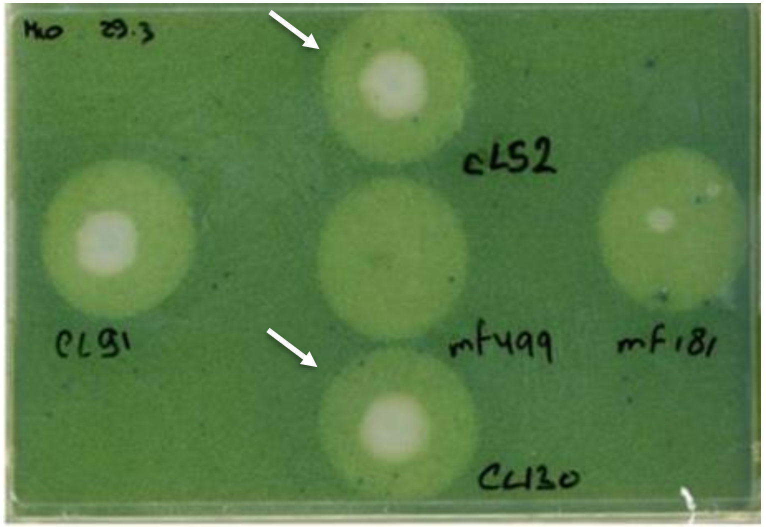
Burkholderia



Pseudomonas



Paenibacillus



The *Burkholderia* MF6 and MF7 virulence over *Chlamydomonas* is contact-dependent

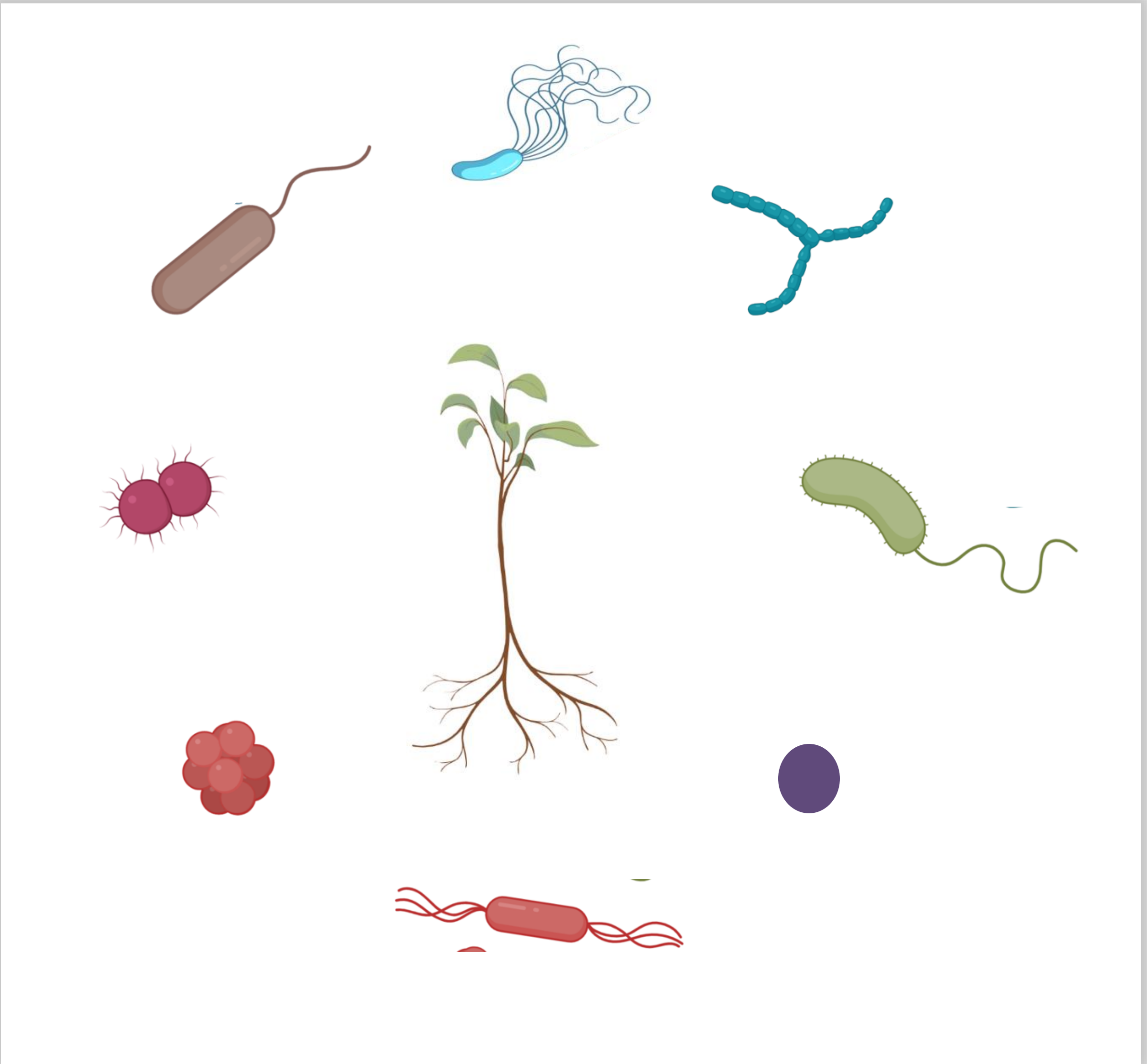
Summary I

- **An experimental setup** for **robust and rapid screening** to disentangle microbial effect over the host

- Host-microbiota interactions are deeply rooted within evolution - **high-conservation of bacterial antagonism** towards plants and microalgae

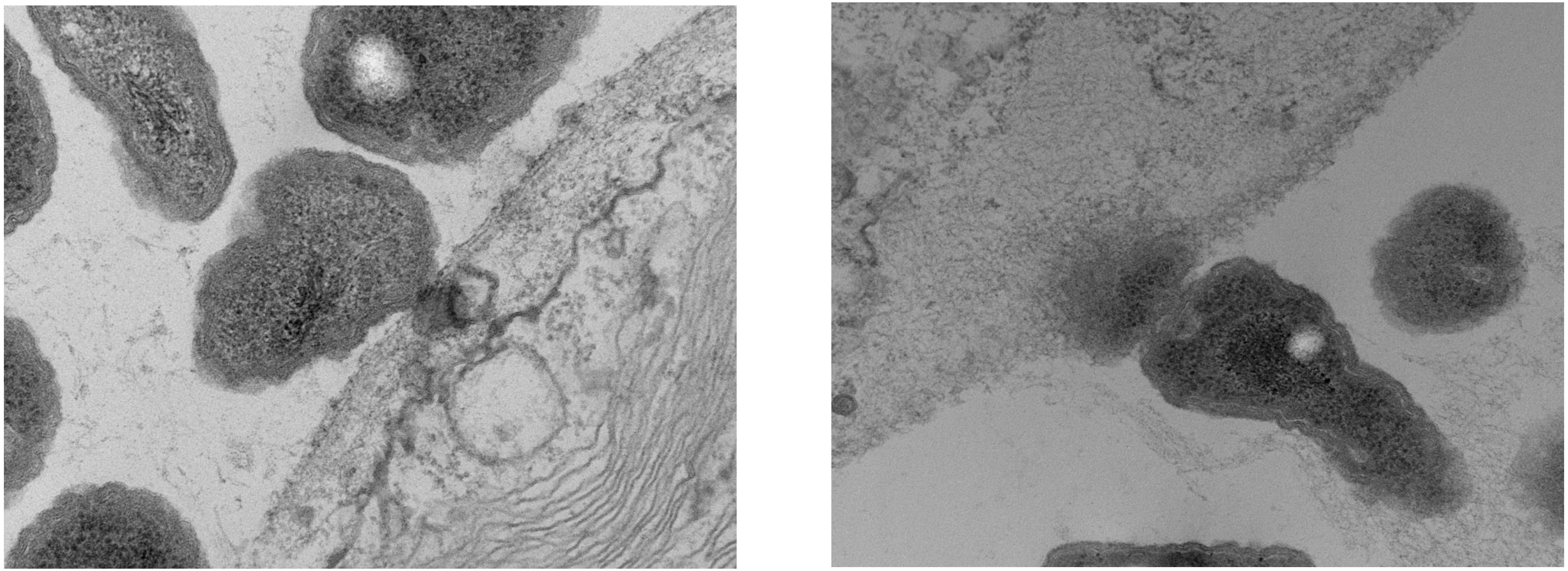
- Antagonistic effect can be mediated in a **contact-dependent** or **independent** manner

- Antagonistic effect may be either **green lineage specific** or with **broader impact**



MF6 facilitates binding and material injection into *Chlamydomonas*

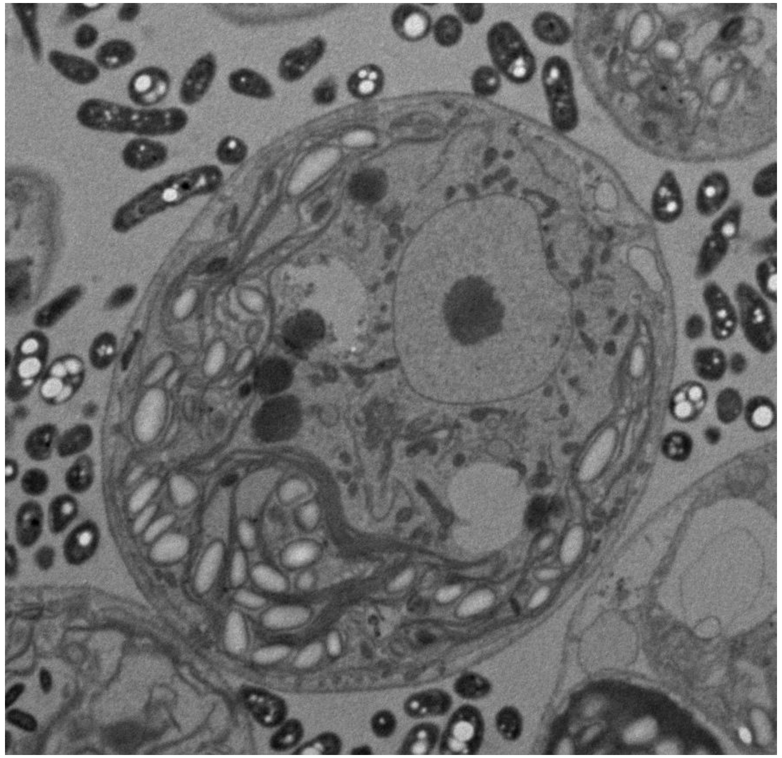
8hr following inoculation



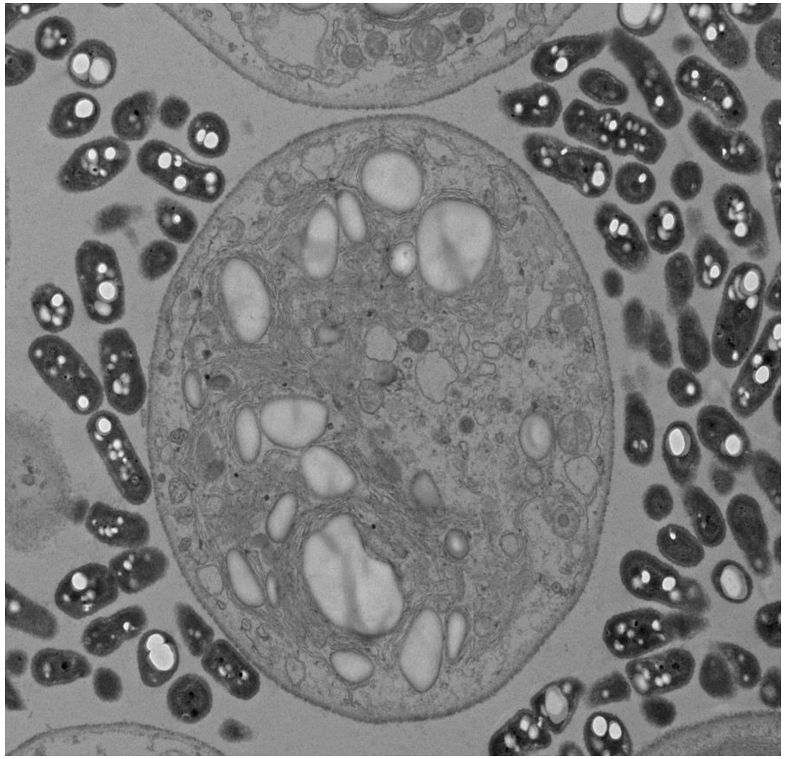
TEM analysis demonstrates autophagic-like cellular degradation

8 hrs

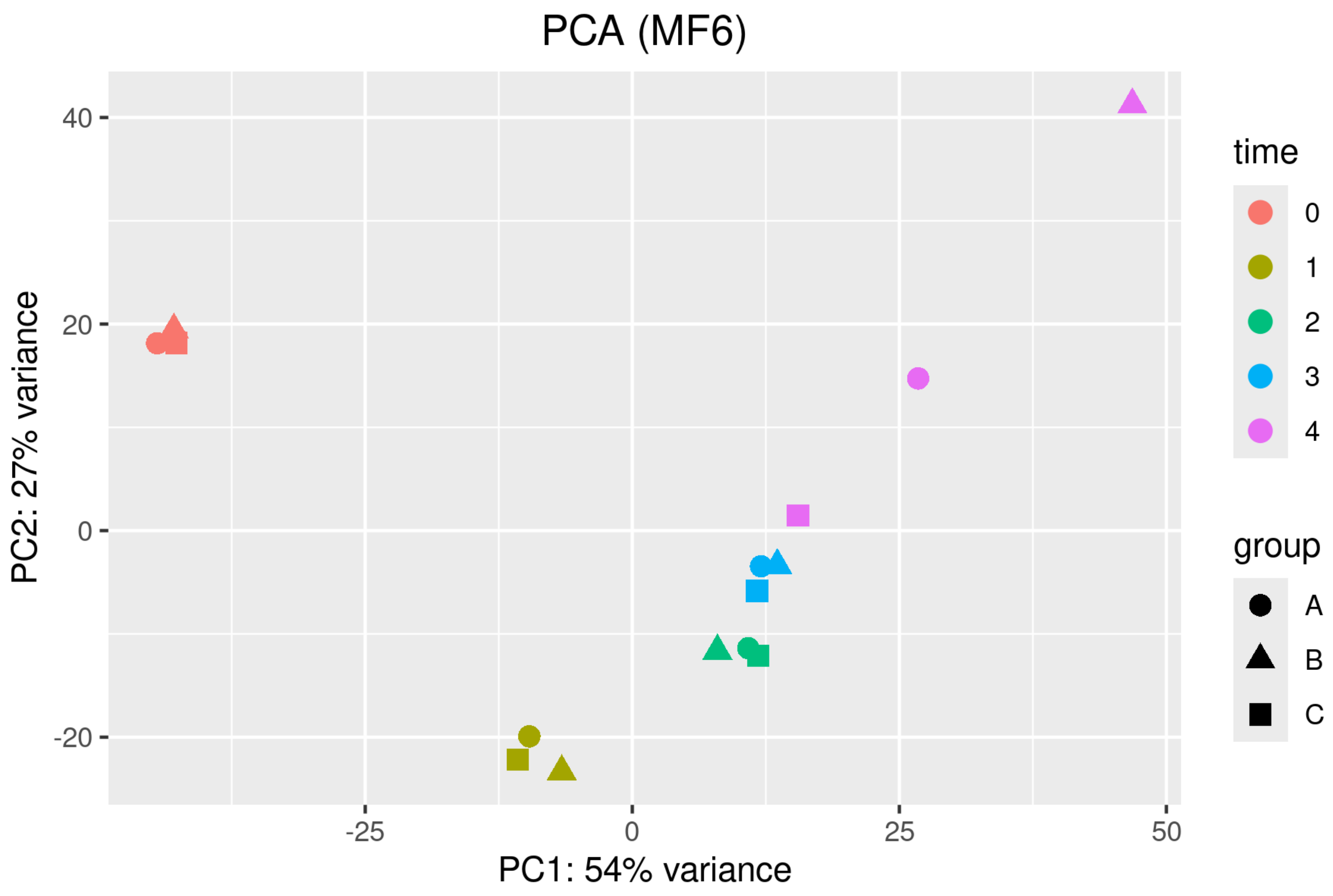
20 hrs



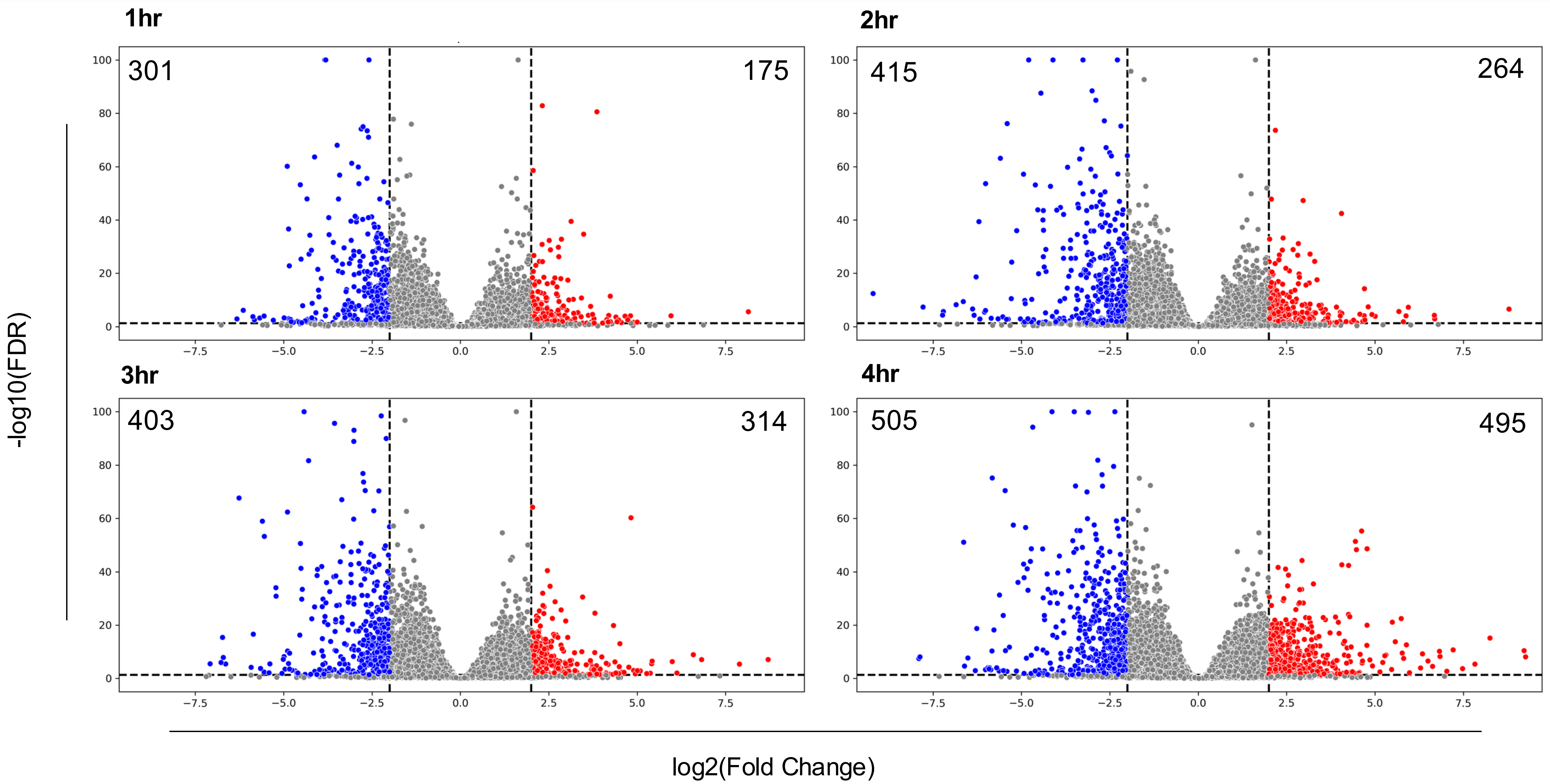
20 hrs



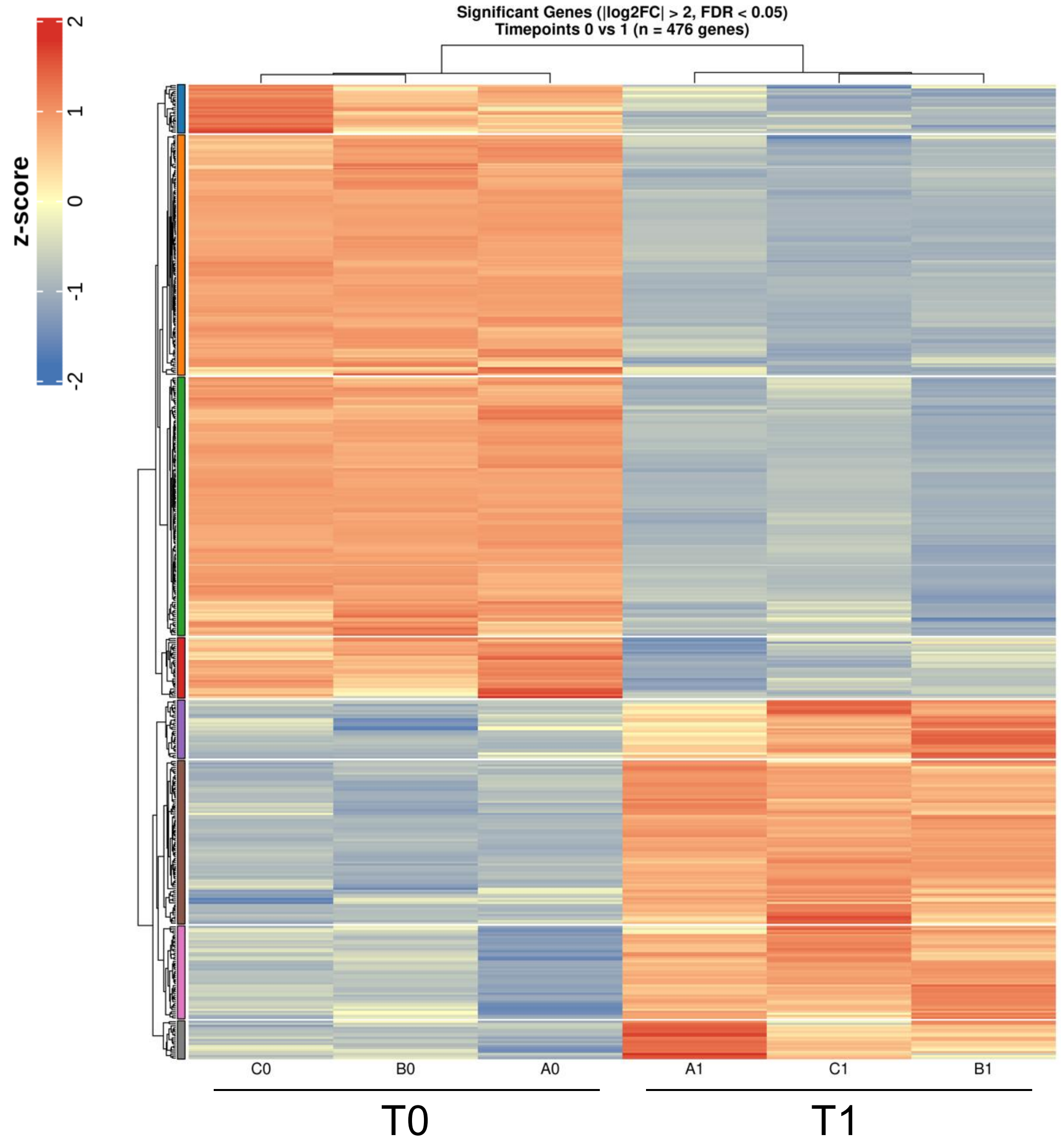
PCA of RNA-Seq data revealing time-dependent clusters



Differential expression landscape over time



1-Hour transcriptome response



Gene Ontology

ammonium **transmembrane transport**

protein phosphorylation, **aa metabolic process**

response to light stimulus, electron transport chain

nucleotide biosynthetic process, intracellular signal transduction

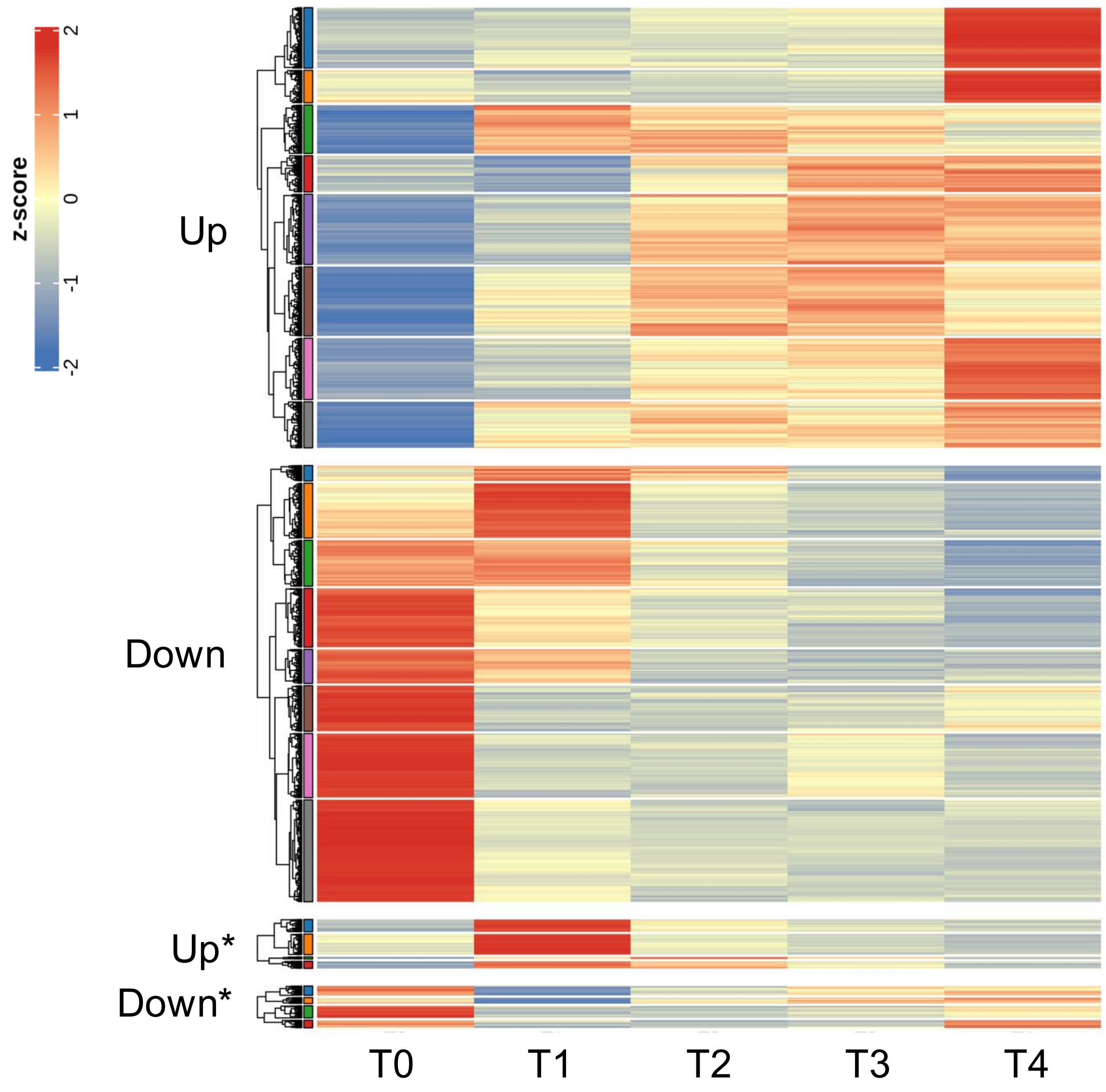
DNA integration, recombination

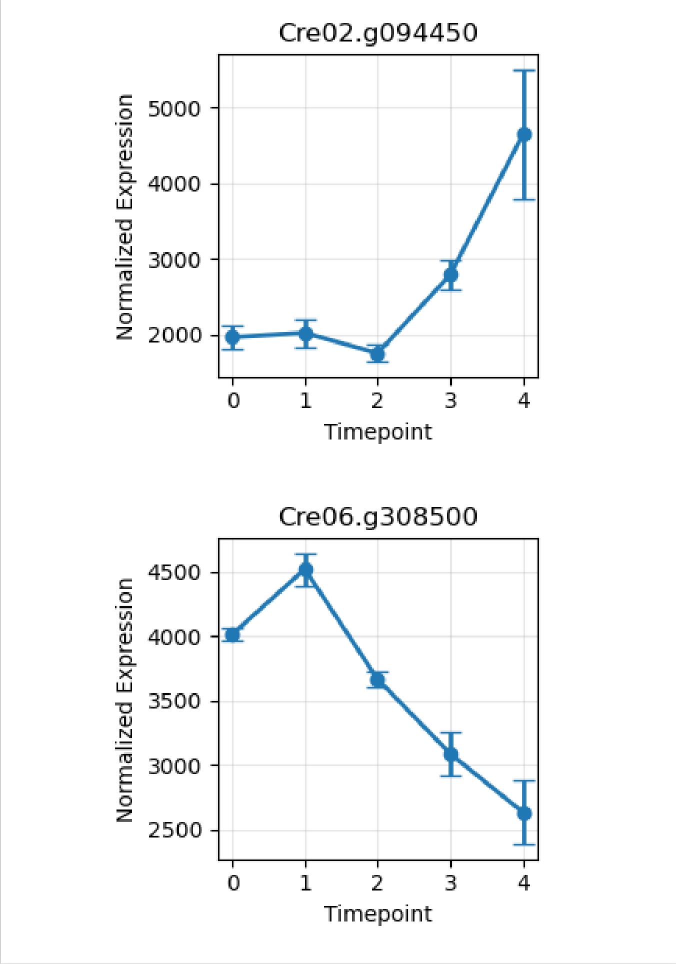
superoxide metabolic process

immune response, **defense response to virus**

phenol-containing compound metabolic process

RNA-seq differential expression patterns over time





rotein ubiquitination
proteolysis

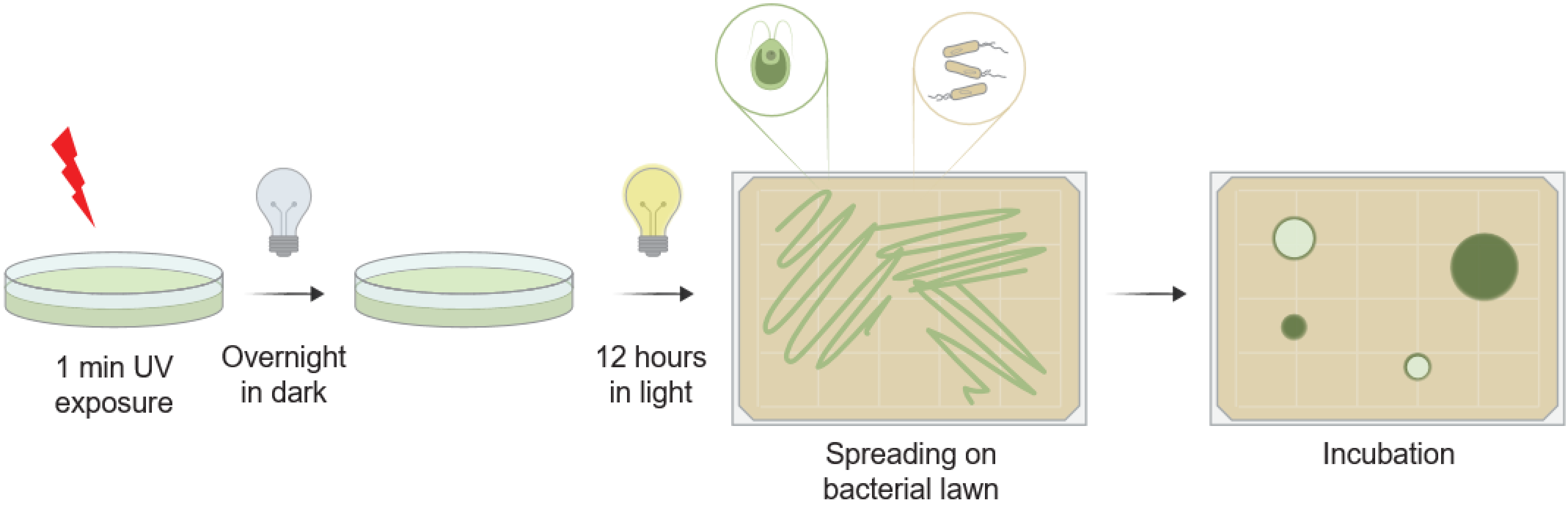
keleton organization

y, proteolysis
ation

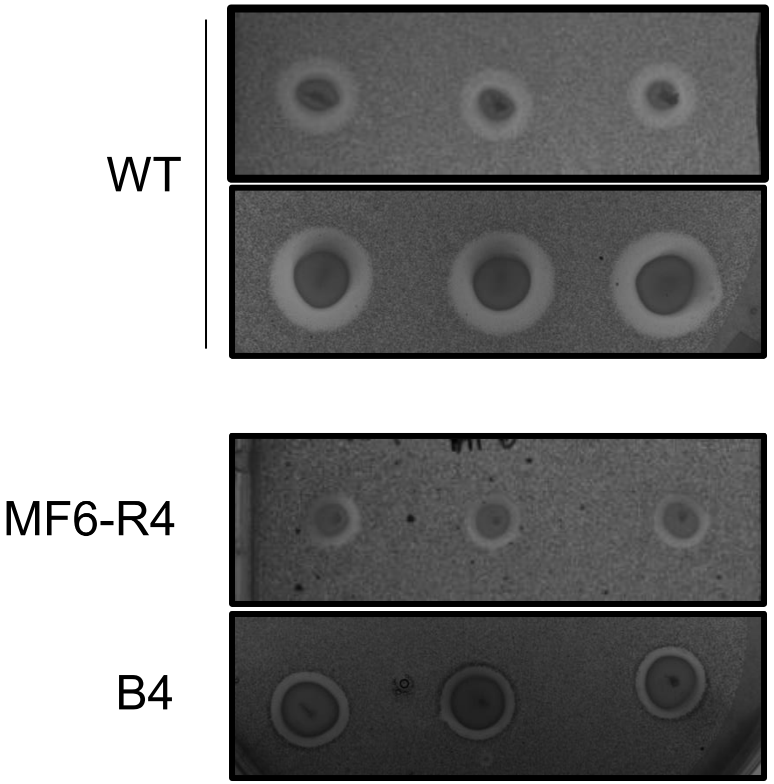
, ion transmembrane transport

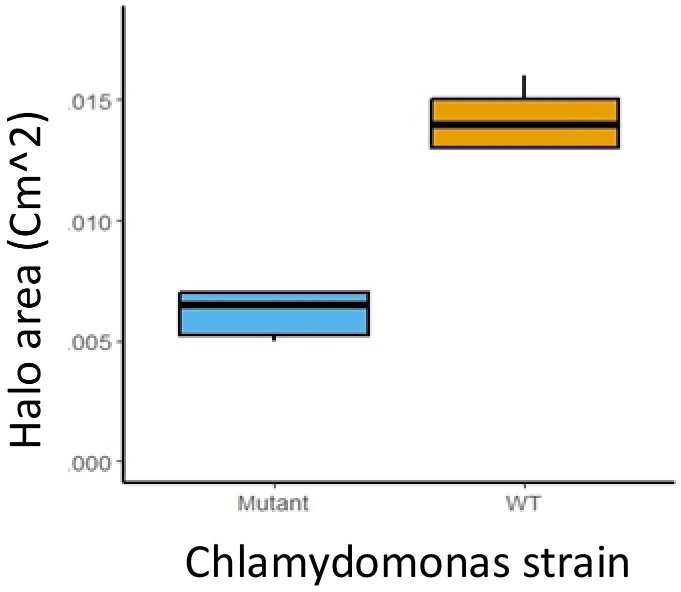
biogenesis

Hunting for resistant *Chlamydomonas* to MF6

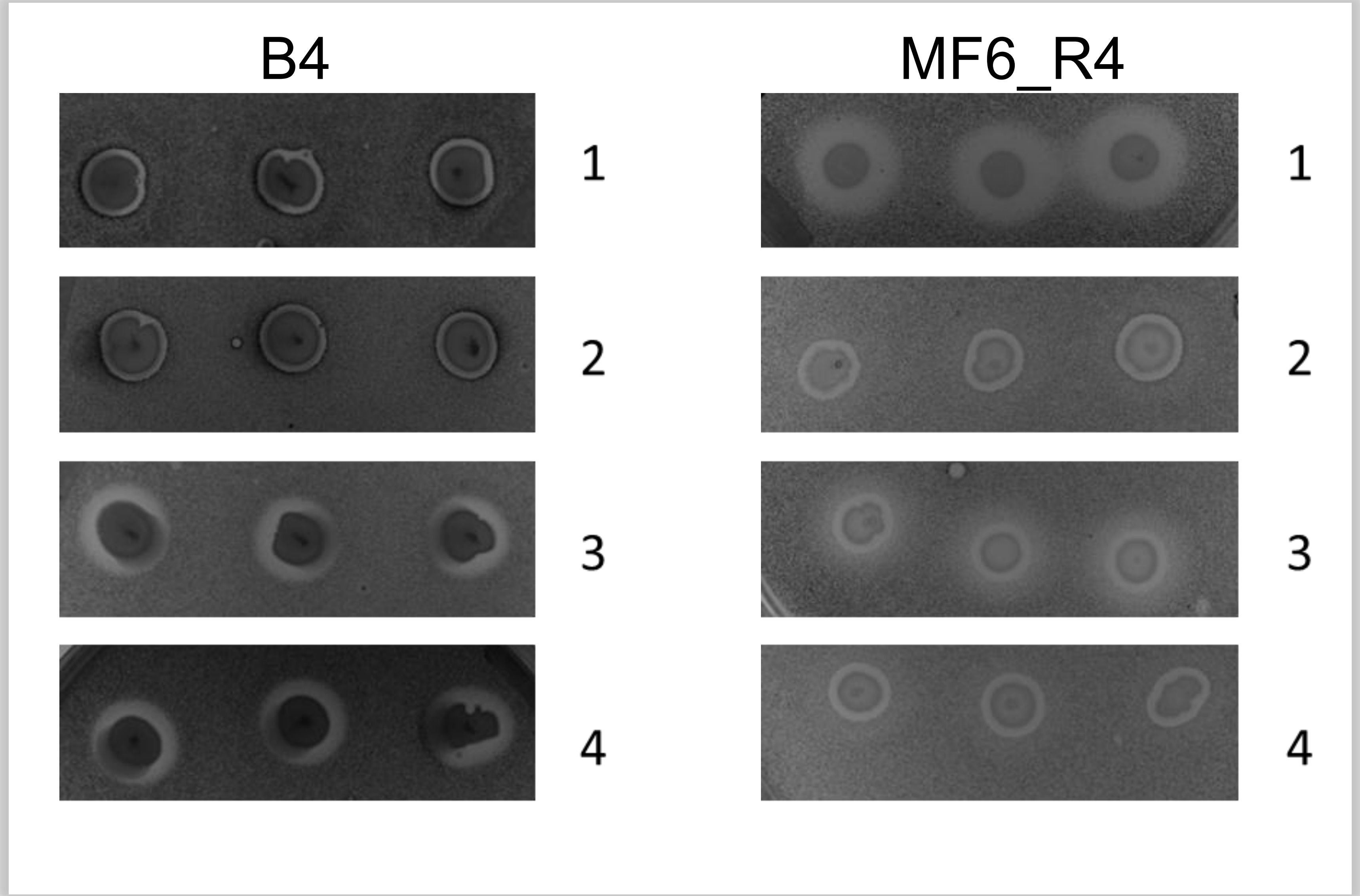


Two resilient mutant strains were isolated

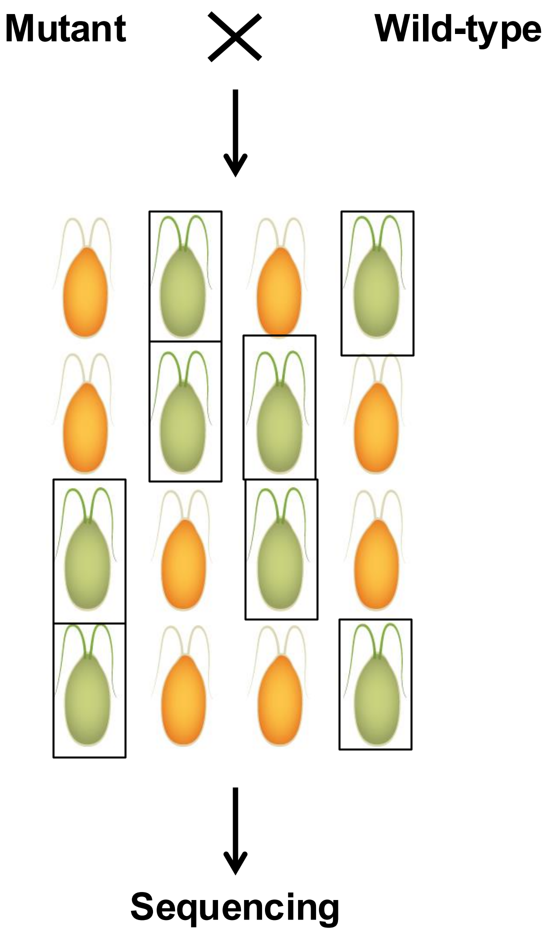


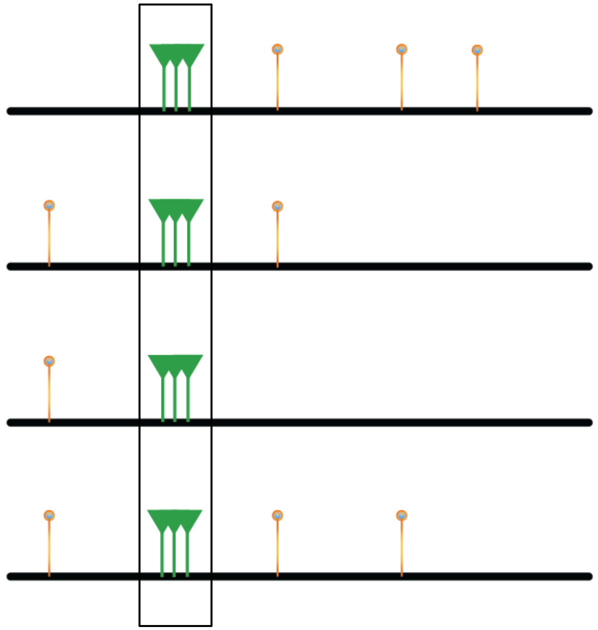


A single genetic lesion underlies resilience to MF6 in both mutants

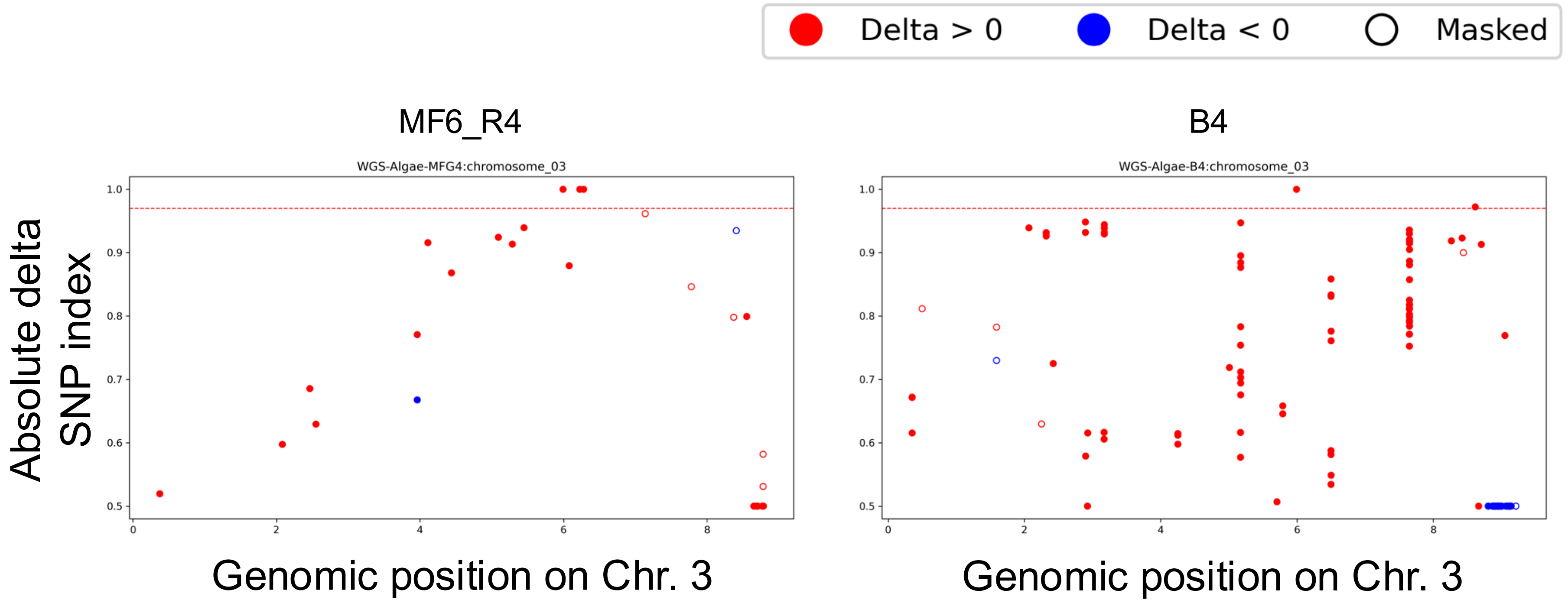


The bulked-segregant analysis is used to discover causative genetic lesions





Two alleles of *zip3* were identified



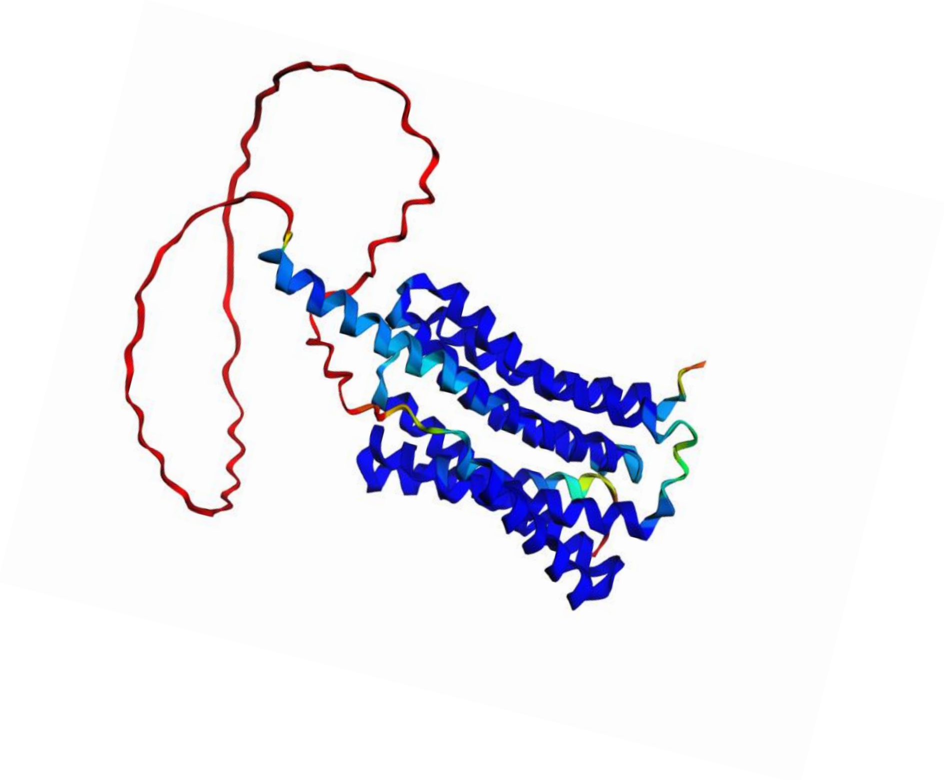
L189P

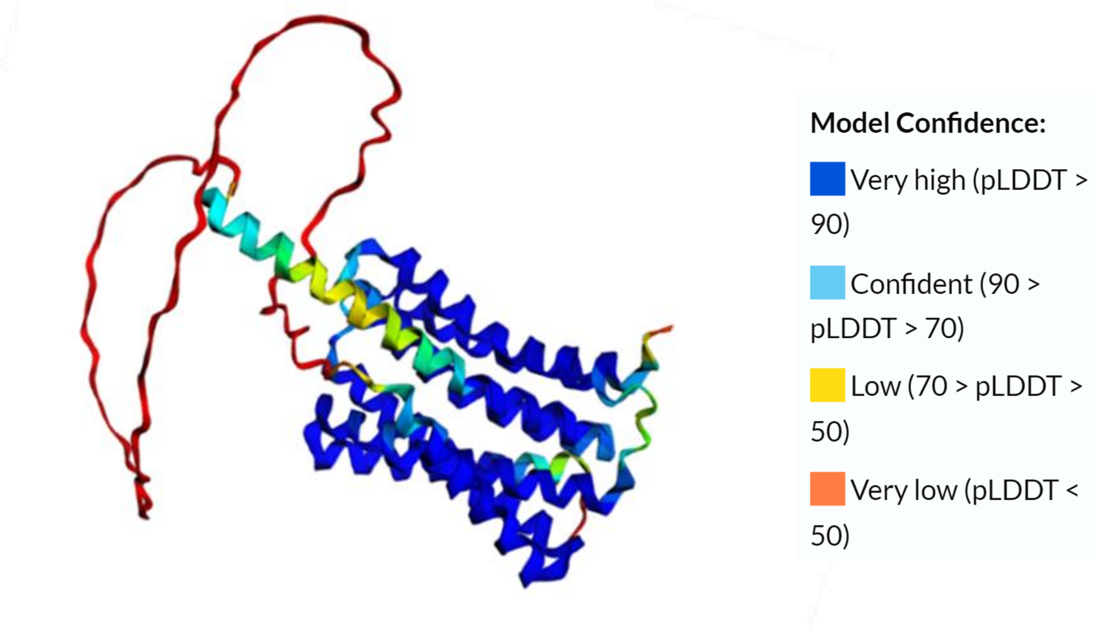
chromosome_3:T5856051C
Cre03.g189550; ZIP3

Pre-mature stop codon in the first exon

chromosome_3:C5855163T
Cre03.g189550; ZIP3

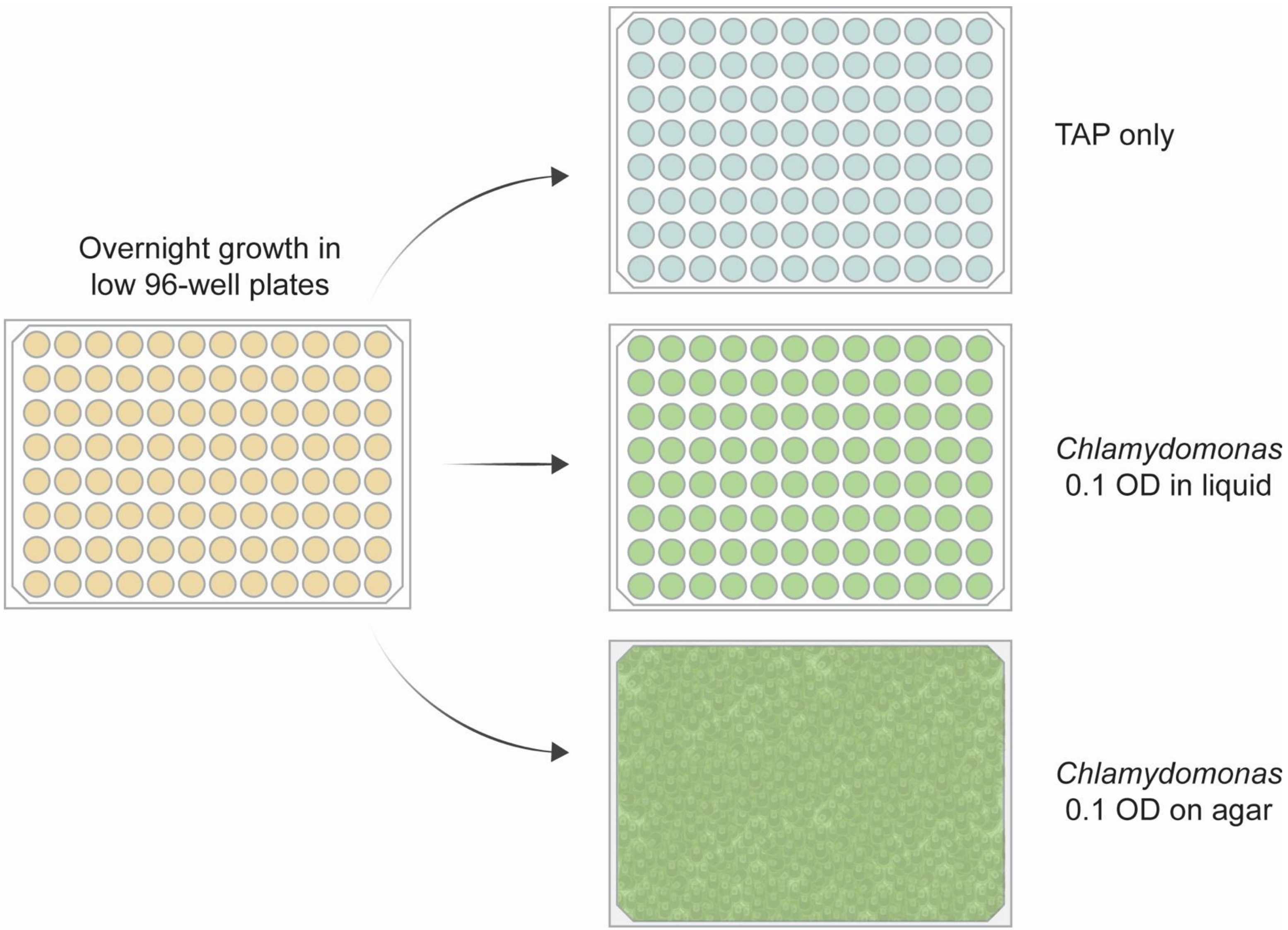
The ZIP3 transporter is predicted to have 8 transmembrane alpha helixes



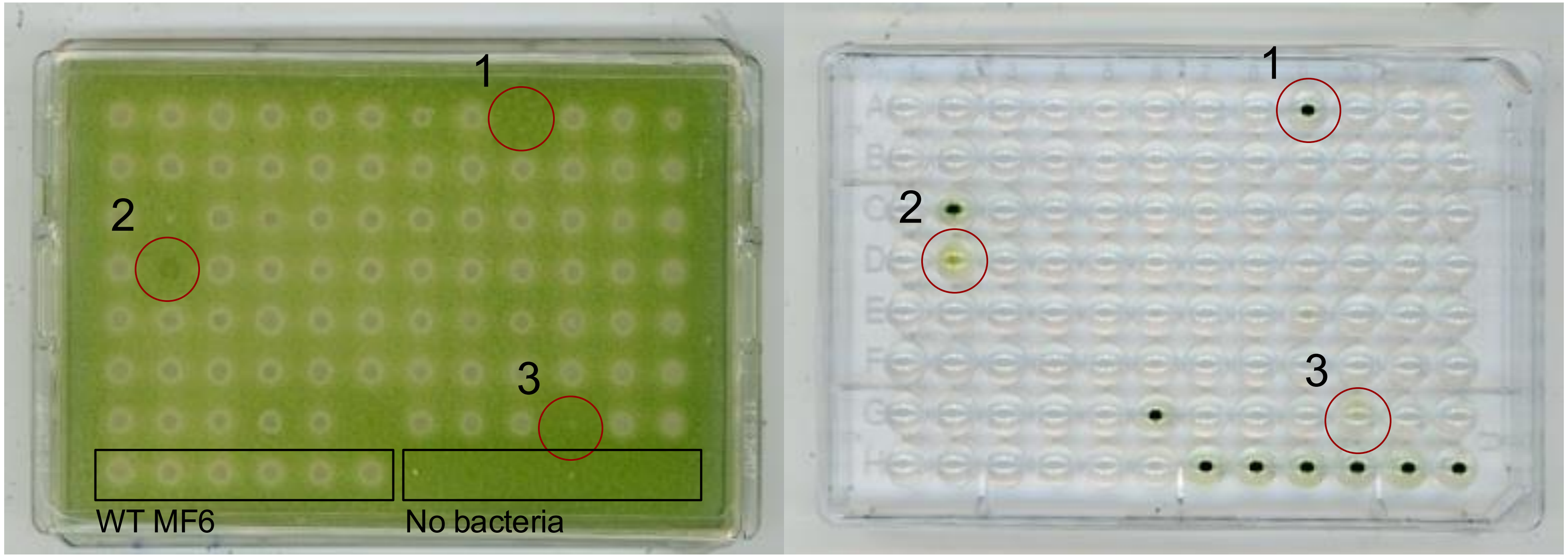


L189P

Generating transposon-based mutant lib of MF6

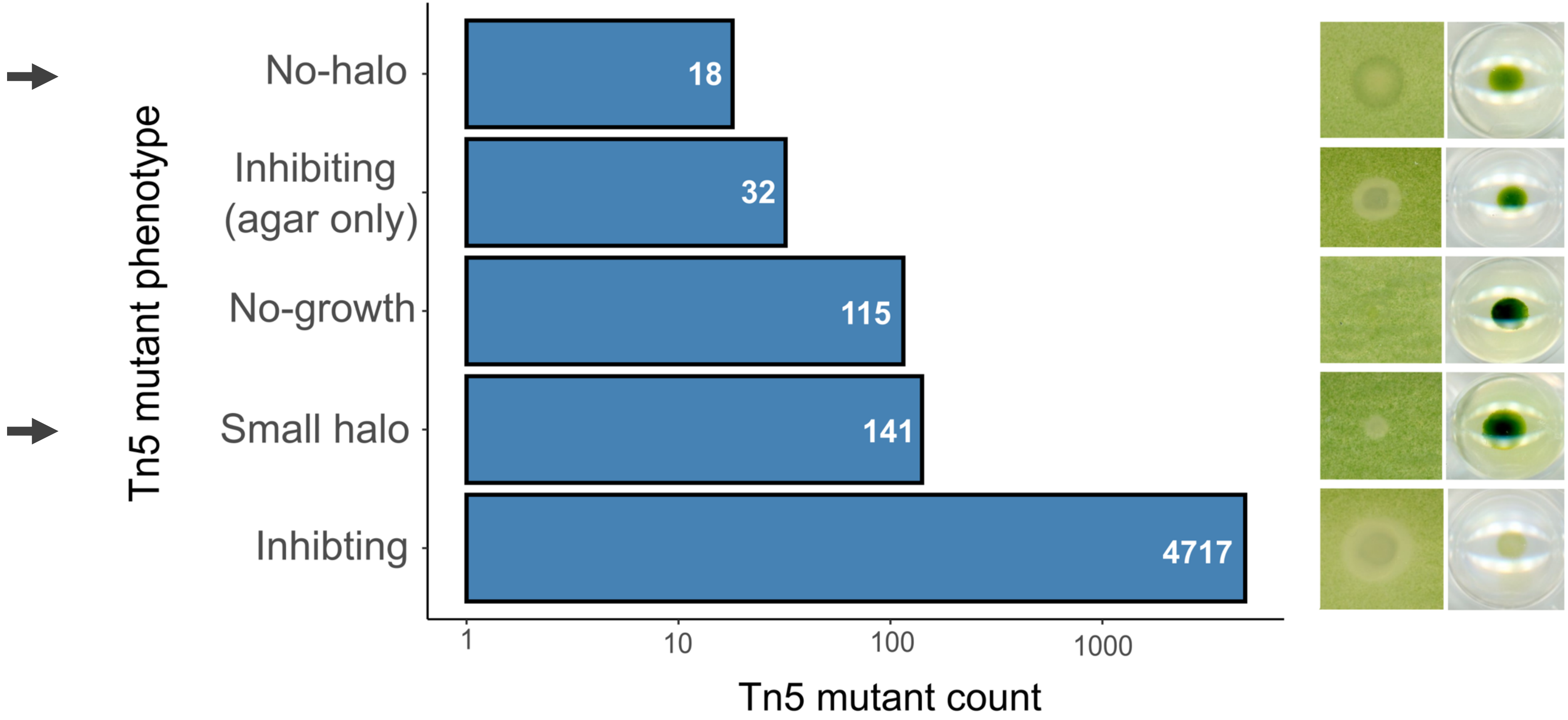


Three main phenotypes emerge



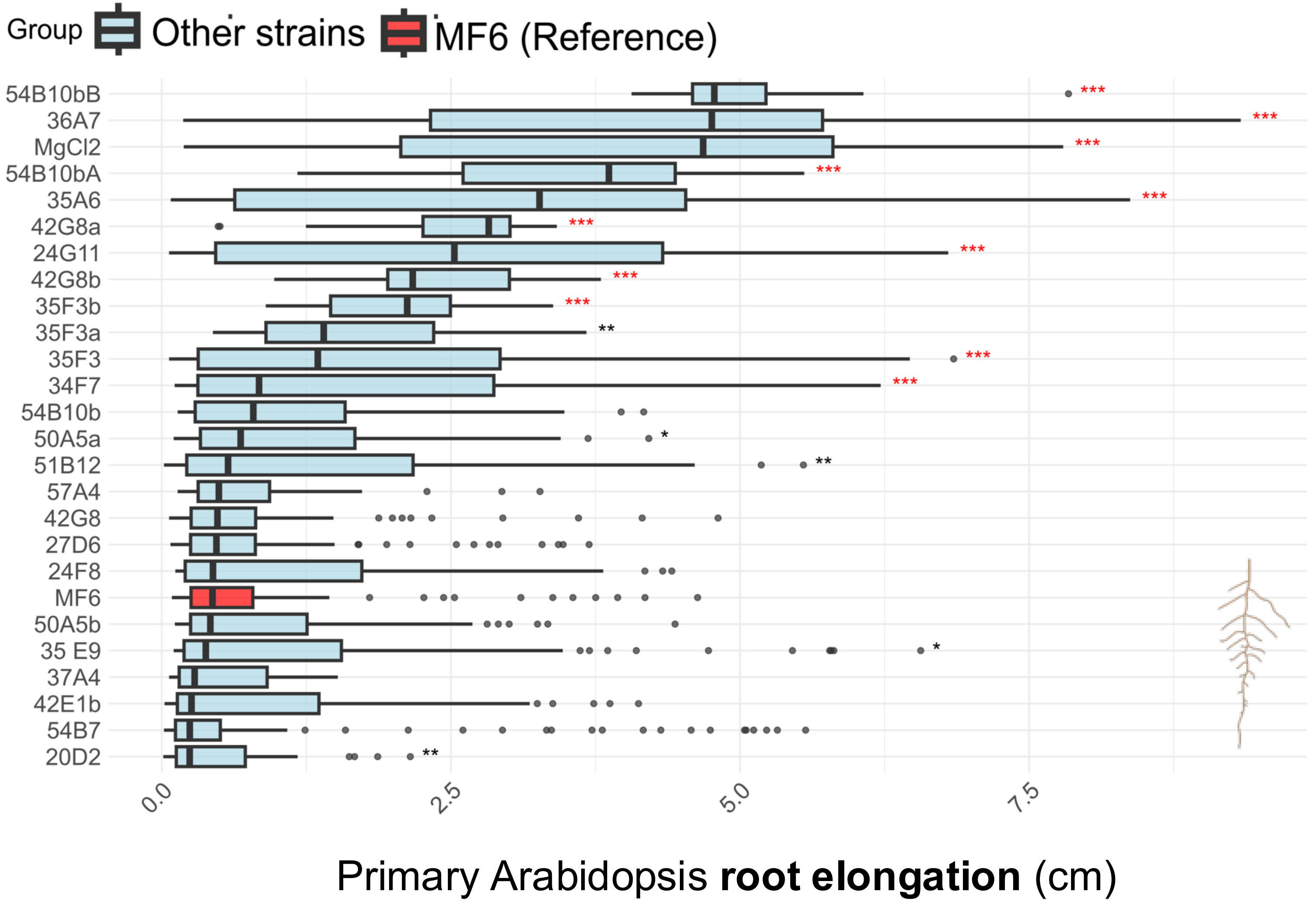
1. No bacterial growth on agar
2. Bacterial growth but no halo on agar
3. *Chlamydomonas* is inhibited in 1 of 2 growth setups

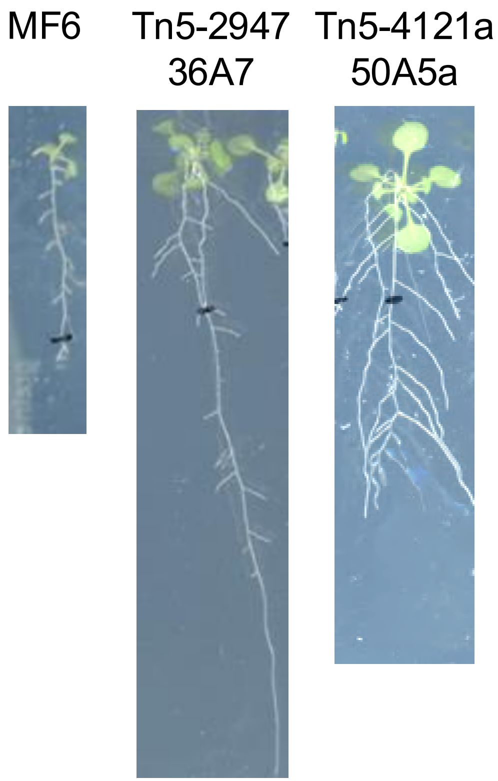
Multiple MF6 mutants lost their virulence



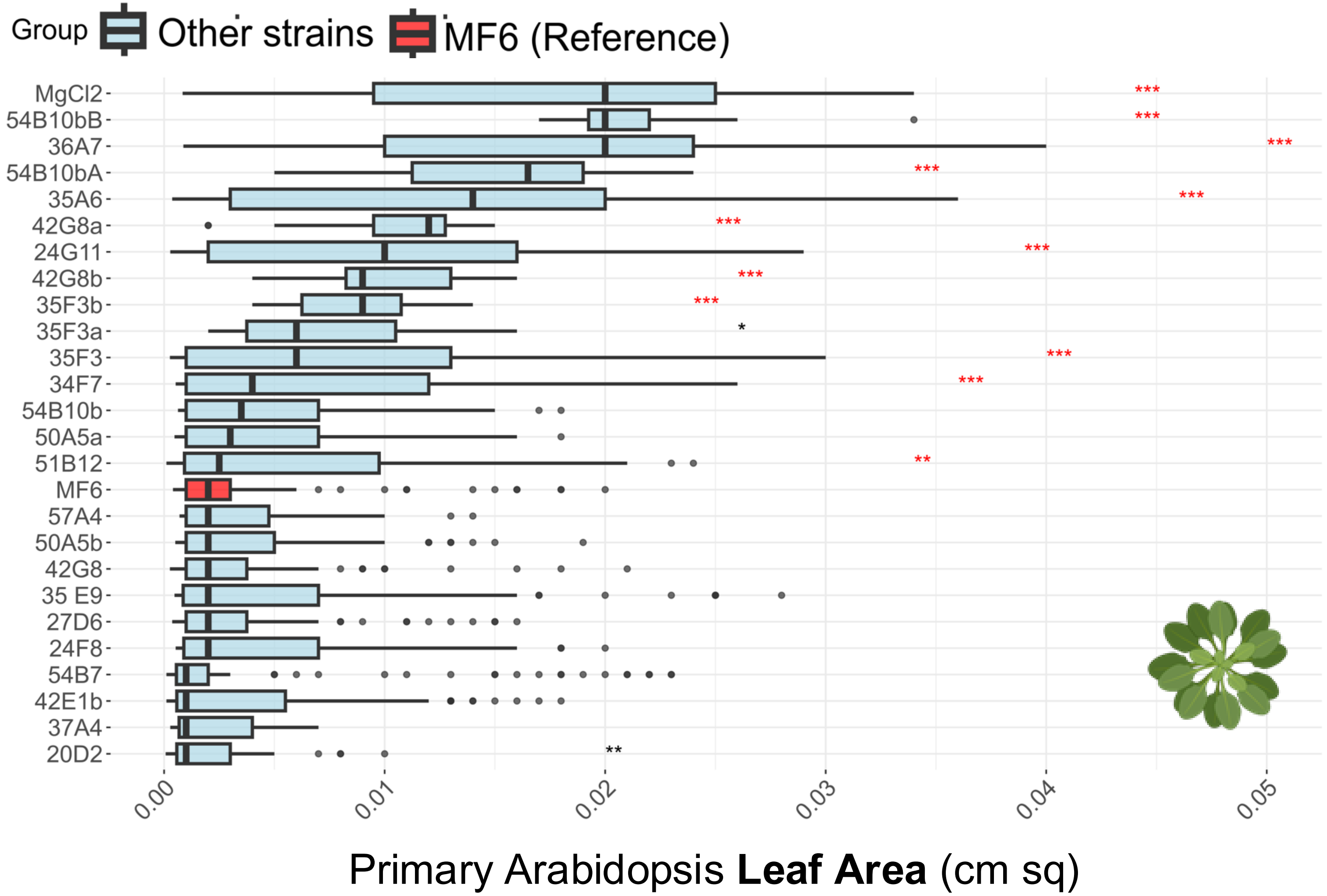
Following secondary screen 18 final mutant strains were picked for further study

At least four different genes are necessary for antagonizing both *Arabidopsis* and *Chlamydomonas*

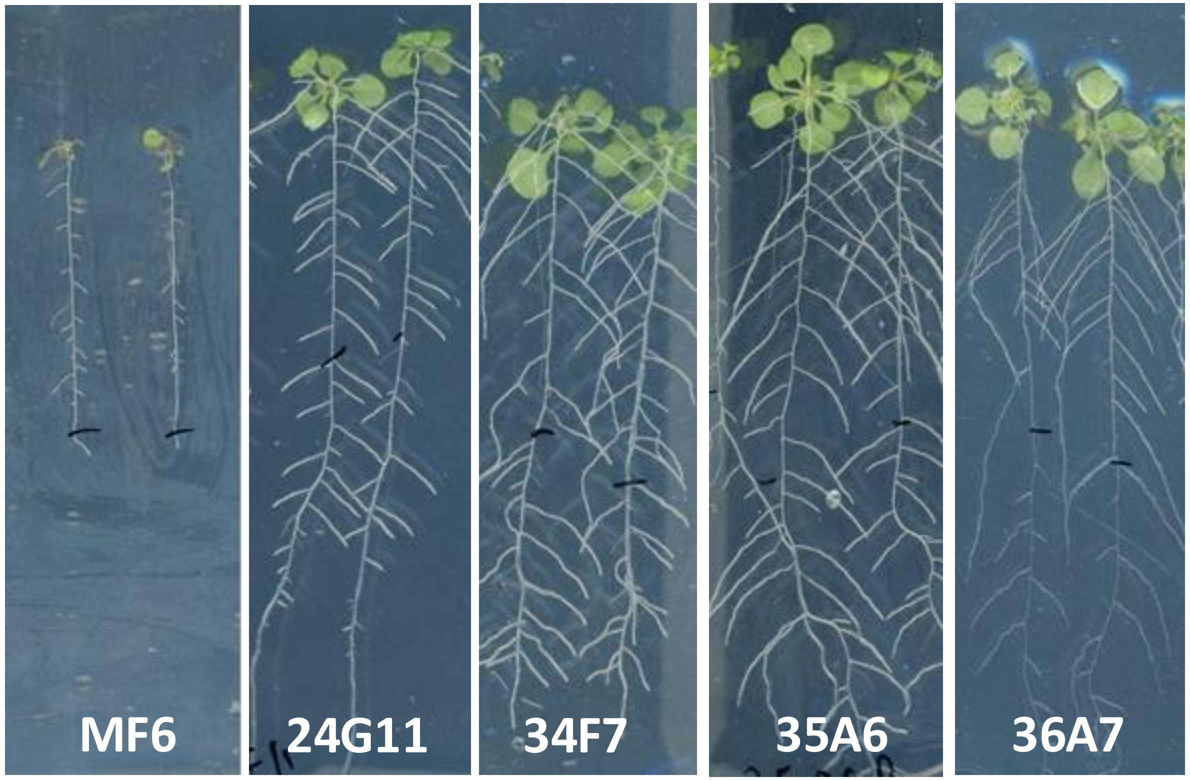




At least four different genes are necessary for antagonizing both *Arabidopsis* and *Chlamydomonas*



Gene insertion sites in MF6 Tn5 mutants



WT

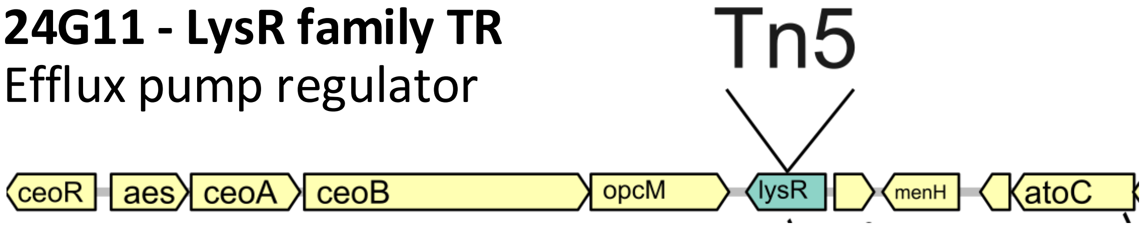
LysR family TR

Rhs element Vgr

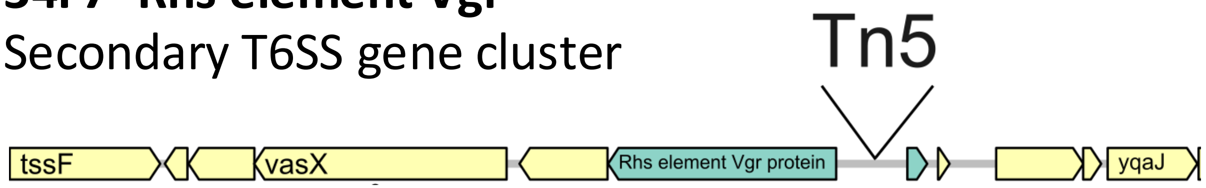
Rne/Rng family ribonuclease

trpE

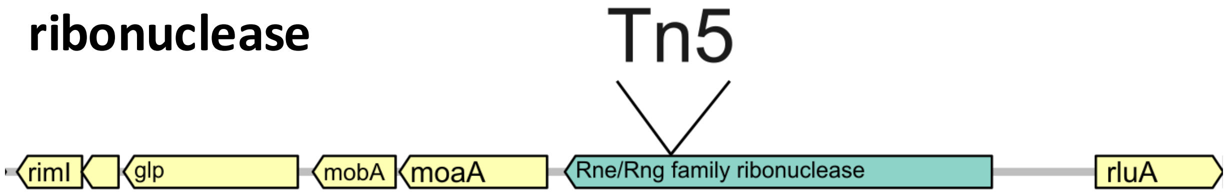
24G11 - LysR family TR
Efflux pump regulator



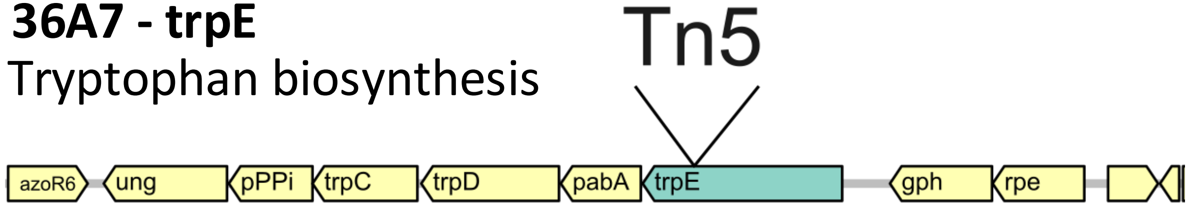
34F7- Rhs element Vgr
Secondary T6SS gene cluster



35A6 - Rne/Rng family ribonuclease



36A7 - trpE
Tryptophan biosynthesis



Summary II

- Utilizing unique genetic and physiological features of *Chlamydomonas* - **powerful and promising model** to address host-microbiome interactions

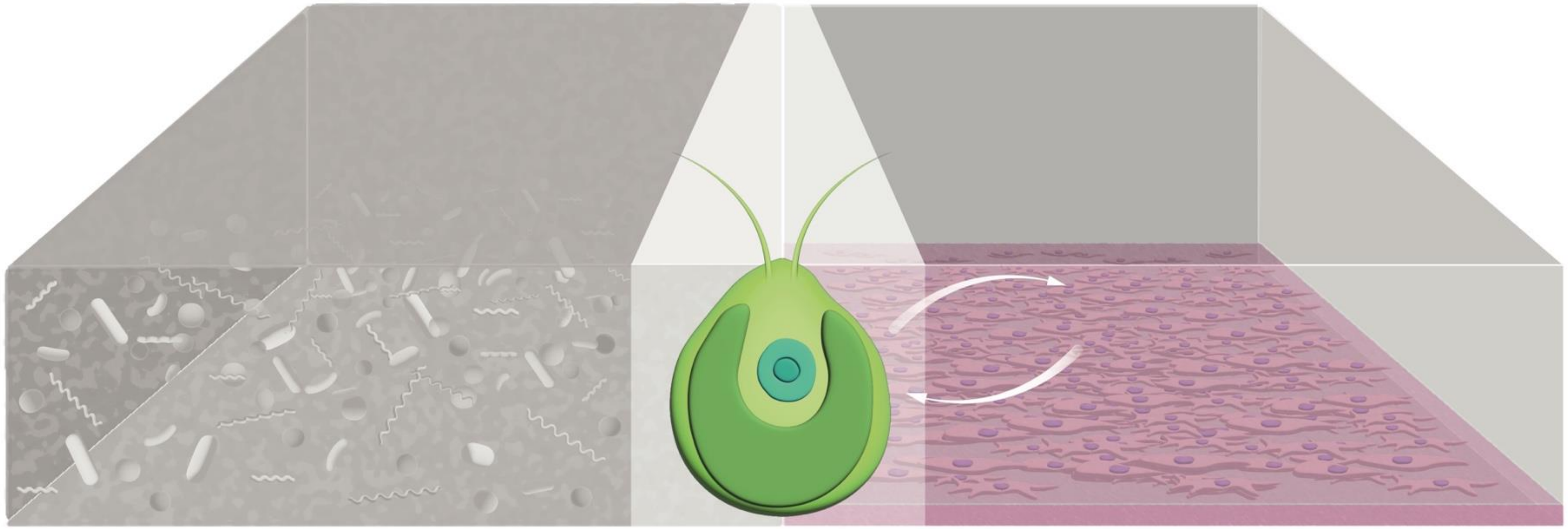
- **An experimental setup** to disentangle microbial effect over the host and the host mechanisms of defense

- Host-microbiota interactions are deeply rooted within evolution - **high-conservation of bacterial antagonism** towards plants and microalgae

- By forward and reverse genetics we can address the **mechanisms** of pathogenicity and host susceptibility

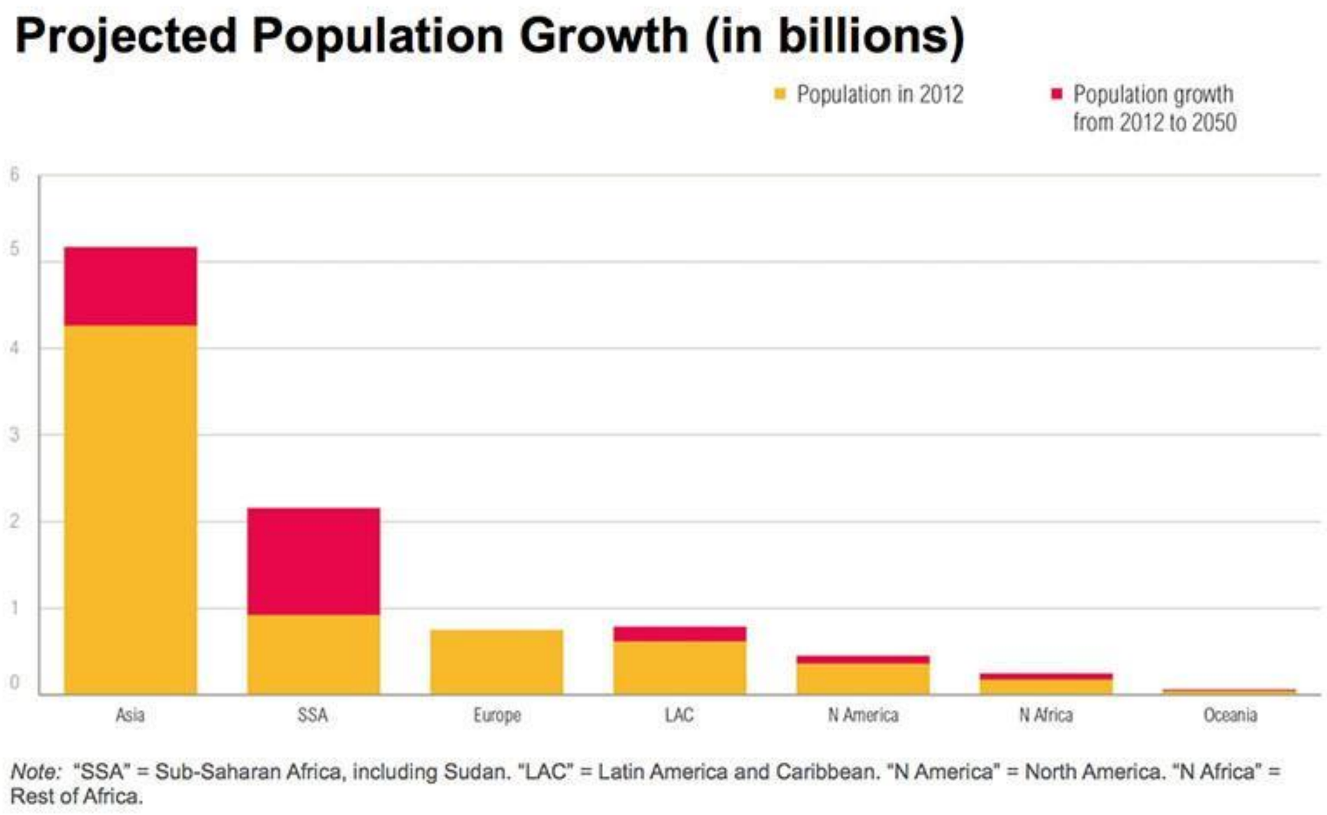
- A **set of bacterial genes** which are necessary for antagonism both for algae and plants

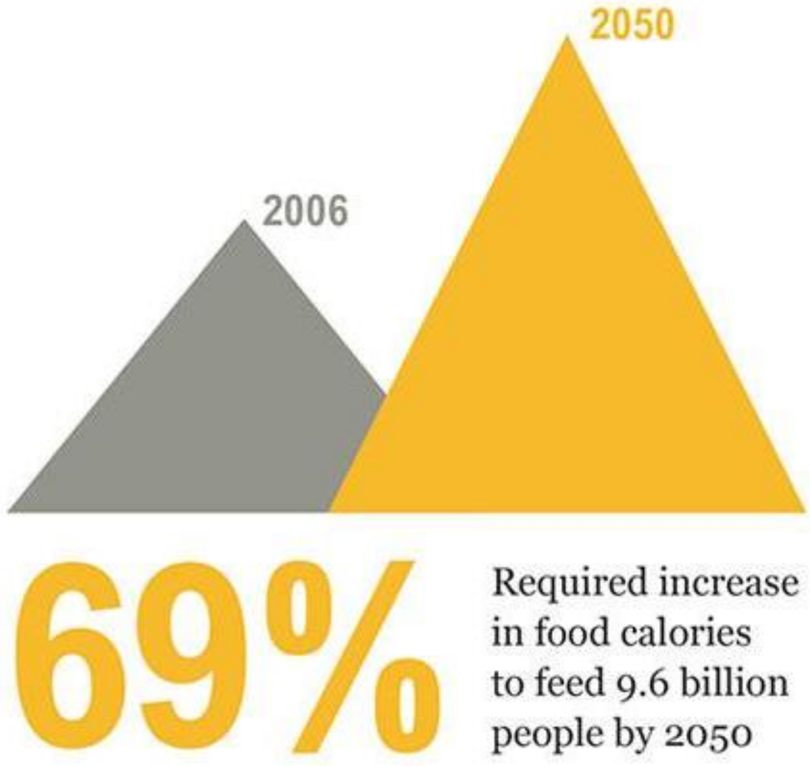
Synthetic microalgae-animal symbiotic ecosystem



Prof. Ofra Benny
Faculty of Medicine, HUJI

World population is expected to reach ~10 billion by 2050



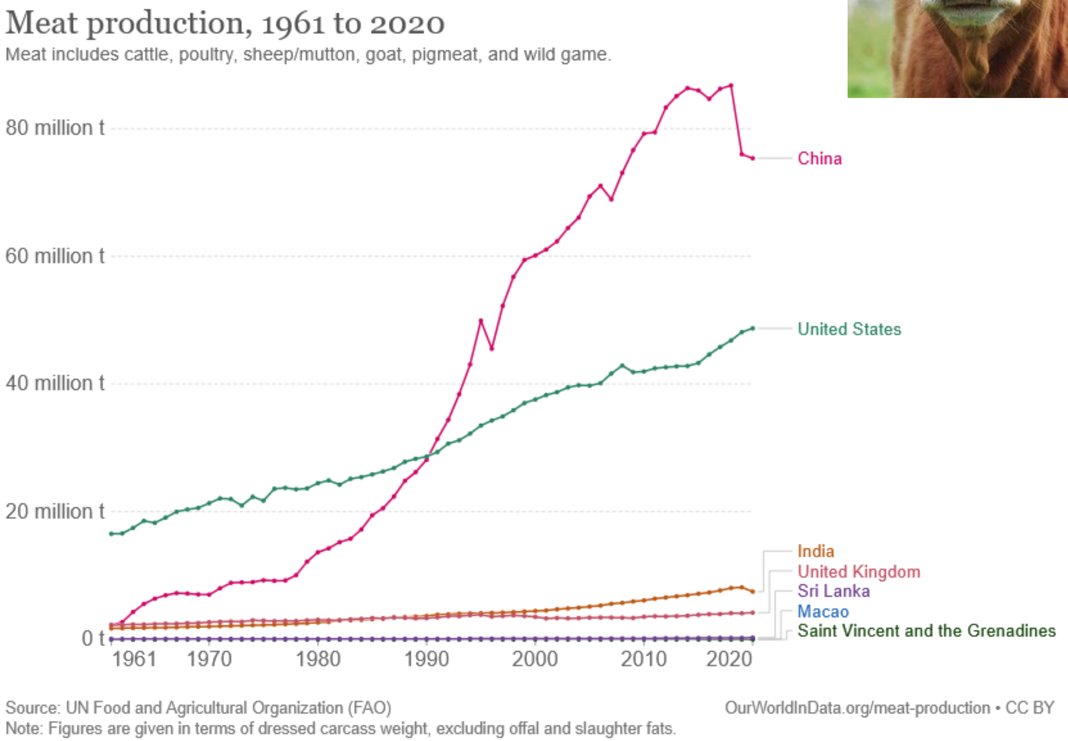


World resources institute

The protein challenge: Feeding people, Sustaining the planet

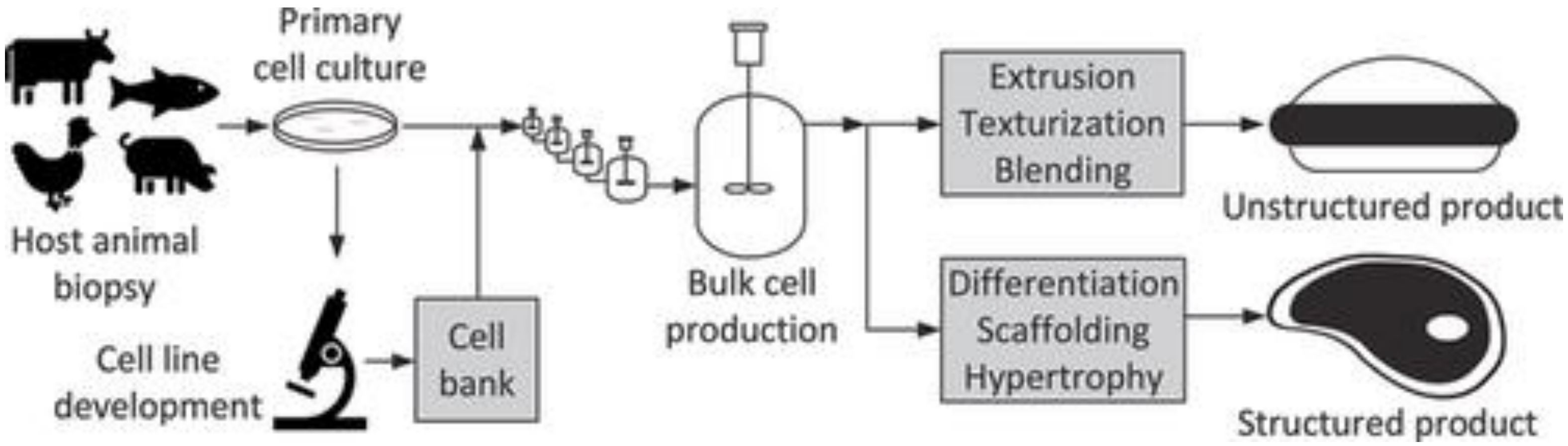
Protein crisis is around the corner with projection of increase in beef and poultry consumption by **66%** and **120%** respectively till 2050

Not only to meet the **increase in demand for meat**, but also to **reduce ecological and environmental footprint** of the expanding livestock sector



ourworldindata.org

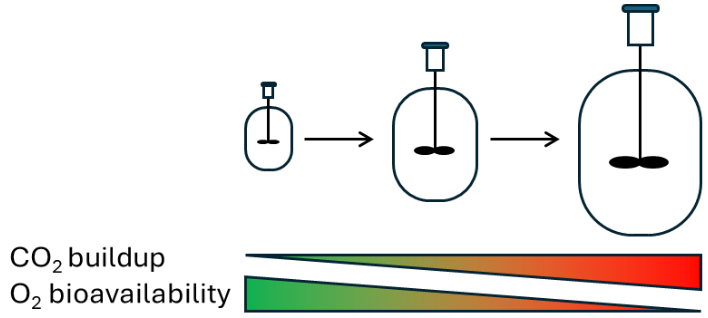
The dream for an affordable Cultured Meat



Ref:Humbird, D. (2021). 118(8), 3239-50.

The dream for an affordable Cultured Meat

- **High production costs.** Growth conditions are extremely expensive: ~2000 $/Kg (estimated 63$ in large-scale production).
- **Scale-up production:** Demand for mass-production to secure food for the growing population.
- **Meat quality:** Demand for a highly nutritious protein

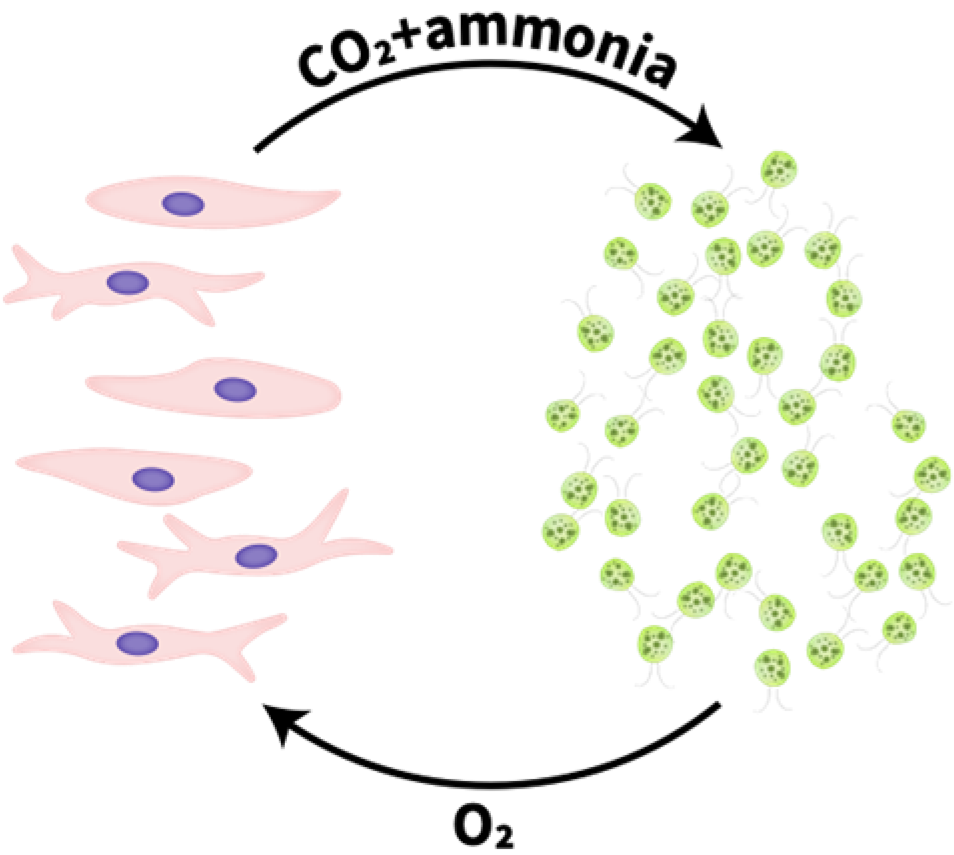


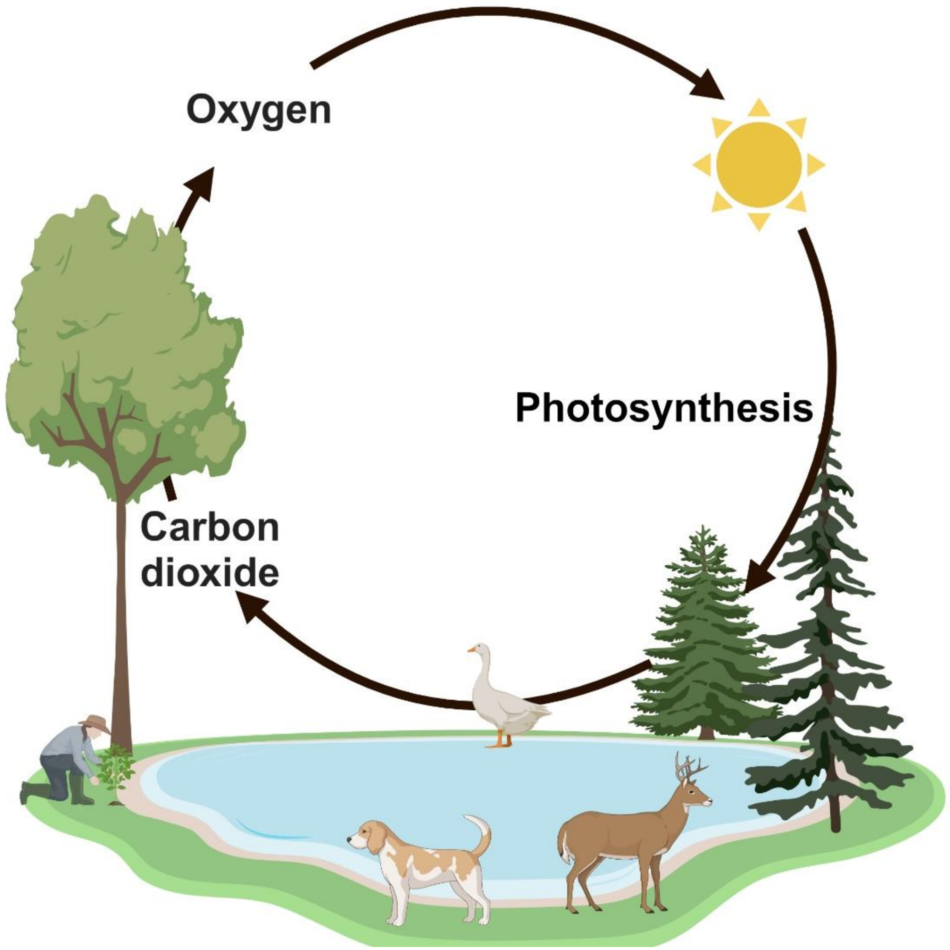
Our Vision:
Crossing the kingdoms

co-culturing of photosynthetic organisms with mammalian cells

Growing an ecosystem

Co-culturing of photosynthetic organisms with mammalian cells.

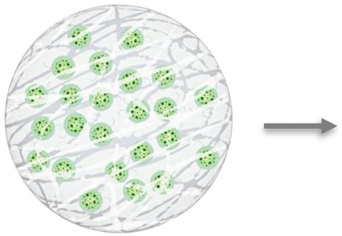




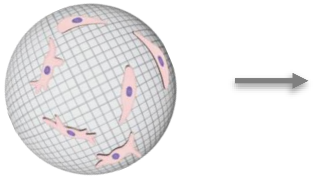
Technology

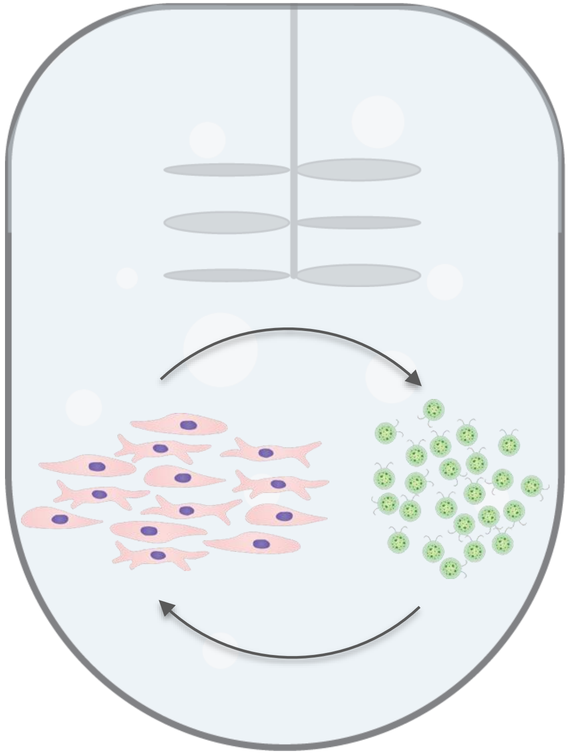
At the core of CultivO2's technology is a dual-compartment co-culture platform that creates a symbiotic ecosystem. The platform consists of two key components:

1. **Photosynthetic microalgae** encapsulated in 3D sodium alginate (SA) matrix



2. **Edible animal cells** growing on microcarriers



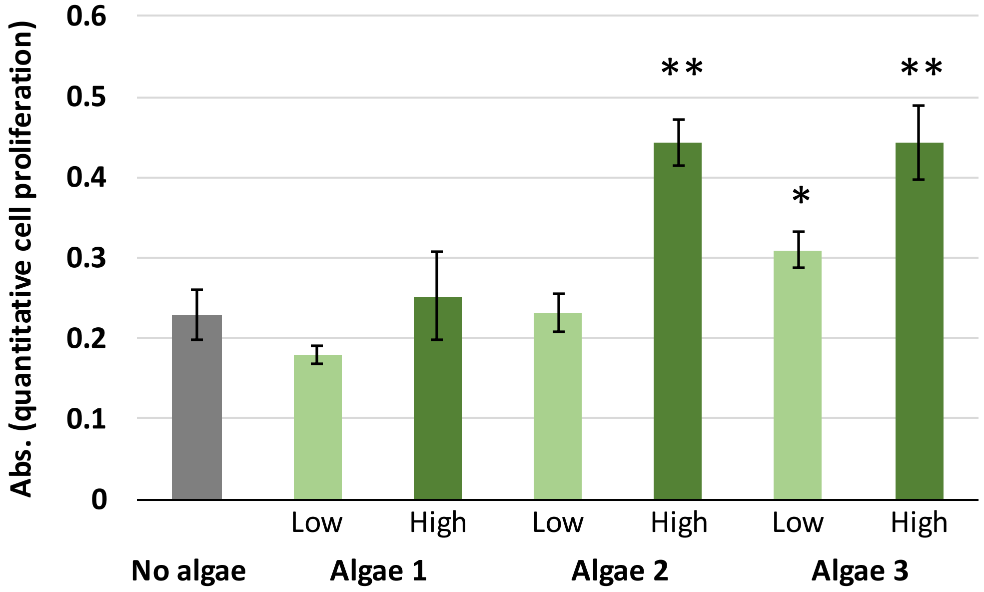


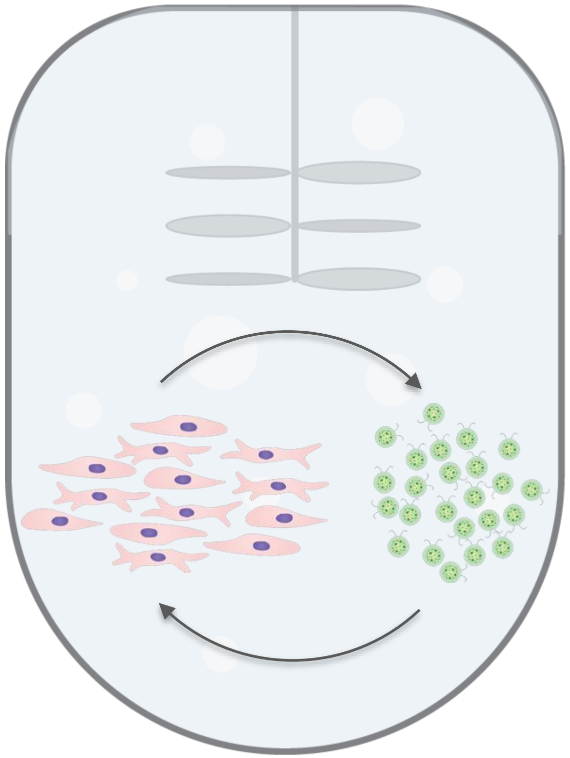
Recycling toxic by-products

Supplementation of nutrients and growth factors

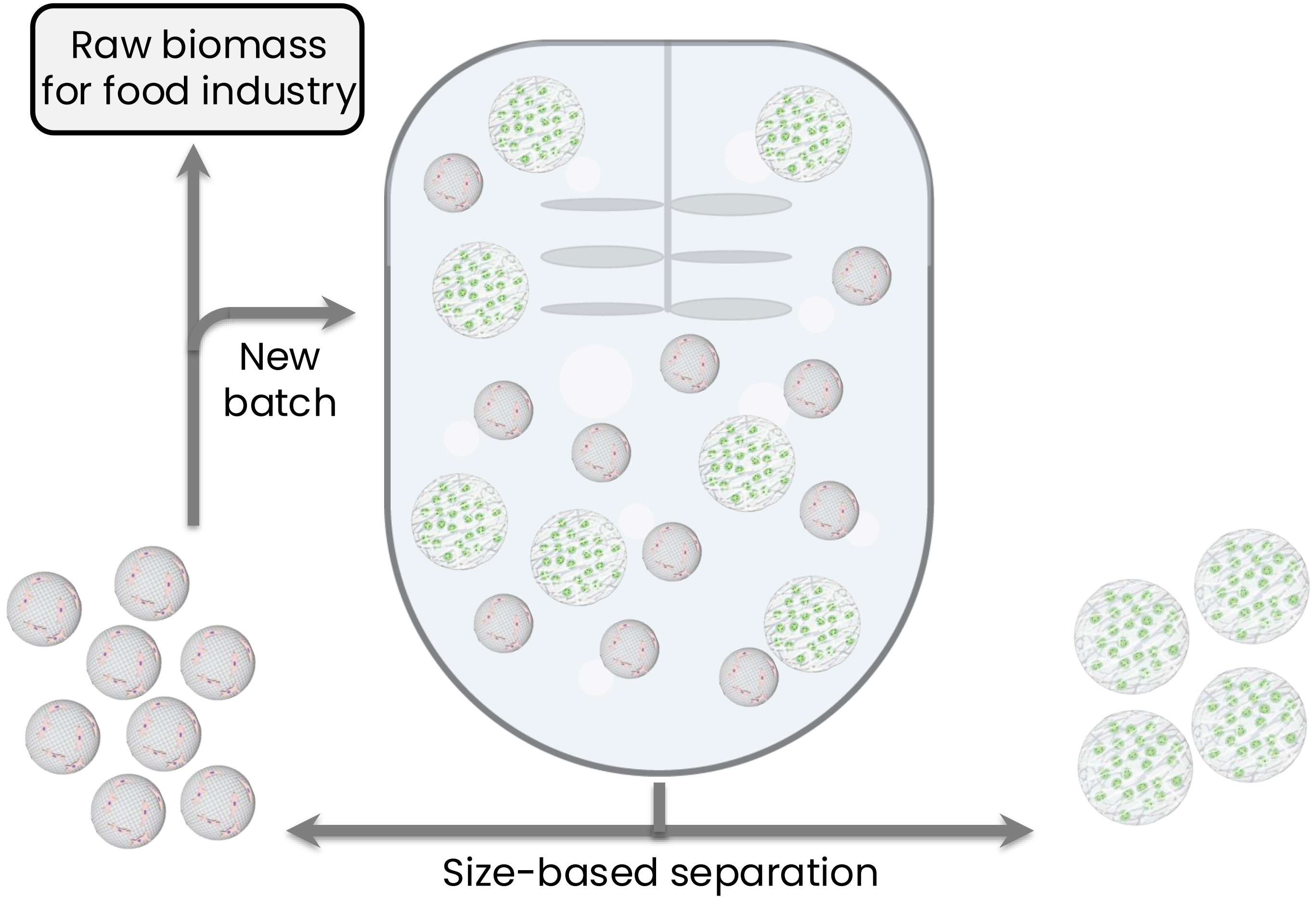
Gas exchange

Microalgae Presence Doubles the Biomass Within Two Days

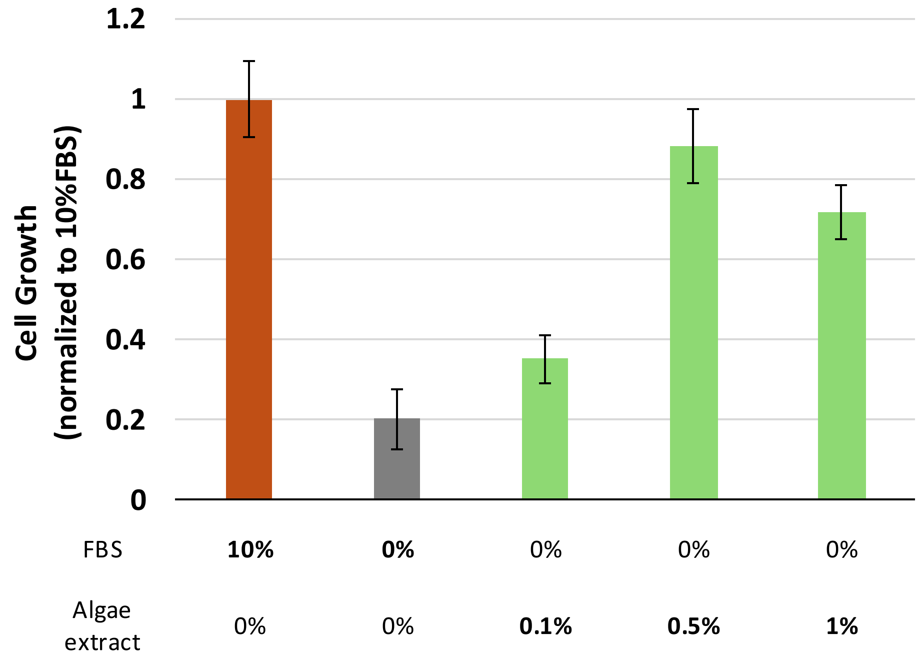




Algae based technologies for cultivated meat

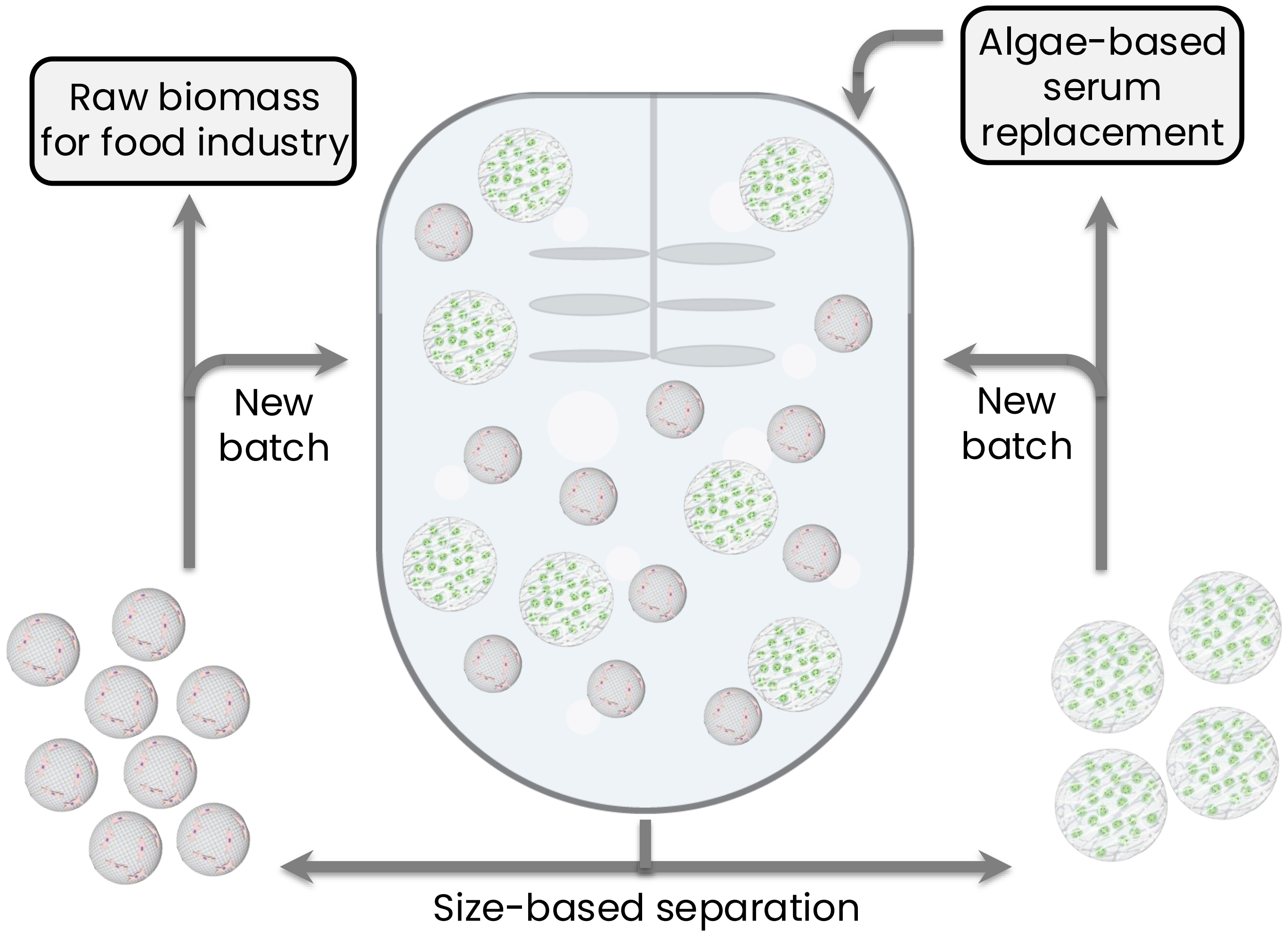


Algae based serum-free media production



We have developed an algae extract that supports the serum-free growth of a cell line relevant to cultivated meat, reaching approximately **90% of the growth achieved under optimal serum conditions**.

Algae based technologies for cultivated meat



CultivO$_2$

innovative food solutions

Summary

- **Bio-convergence** accelerates **innovations**
- **Ecosystem based** solutions is a disruptive technology that can bypass central hurdles in cultured meat production pipelines
- **Adaptive** for different cell lines (chicken, bovine, fish)
- The road is long, it requires patience, persistence and **vision**

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Israel Innovation Authority

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

מרכז האוניברסיטה העברית לקיימות
The Hebrew University Center for Sustainability
مركز الجامعة العبرية للاستدامة

CultivO2

עבודה שלי על ההרצאה הזו:

1. דינמיקת טורף-נטרף ומחשבות על ההריג של MF6
2. לחזור למאמר של עומרי
3. להסתכל למאמר של פרידר בנוגע לפלג׳לה
4. מה הגנים האנטגוניסטים רק כלפיי קלאמי?
5. דופן תא של קלאמי לעומת ארבידופסיס
6. תגובות חיסון יותר מעורבות כלורופלסט
7. רותם סורק – מטאלופרוטאזות
8. מחקר של chory
9. כמה זה באמת מייצג את החיים?
10. האם יש תכונות מיוחדות בחיידקים שאנחנו מעמיקים בהם שכדאי שאני אדע? גאו[illegible]
11. כל המאמרים שנשלחו בקבוצה
12. למה אנחנו חושבים שקלאמי הוא מודל טוב
13. מה המשמעות האקולוגית של העבודה ? MF6?
14. האם מתוך 16S זה מעניין להסתכל על קב׳ חיידקים דומיננטיות כלומר מי בכלל נמצא שם?
15. למה אנחנו חושבים שזה חלבון ולא מטאבוליט
16. האם יש עוד דוגמאות בטבע שבורקולדריה הוא תלוי מגע?
17. איך אנחנו מסבירים את הדינמיקה של MF6-chlamy?
18. כמה זה נפוץ בורקולדריה?
19. שיעור של עומרי בקורס מבוא
20. להרצות מול יעל ואהרון קפלן
21. לקרוא על טרנספורטרים ולחשוב עוד קצת על תוצאות רנ״א
22. מאמר של גרידו, reviewשל ג׳ף, review של אבולוציה עם מיקרואורגניזמים
23. לחדד בטקסט שלי ואולי גם בשקפי הסיכום עם הרעיונות האבולוציונים

מה ההבדל בשנים האבולוציוני

MF6איזה סוג חיידק זה

כמה זה נפוץ בטבע החיבור הישיר?

האקסודטים של קלאמי שונה מאוד משל צמח (לדוג פולט גליקולאט במחסור פחמן דו חמצני). איך בכל זאת אנחנו מסבירים את הרציונל של השימוש במודל?

כמה זה נפוץ שנמצא את נשיקת המוות או בכלל שדבר כזה קורה בטבע?

בניסוי פילטרים למה גדלו קלאמי על הפילטר? הדיפוזיה יכולה להיות מגבלה, יש עניין של ריכוזי חומרים ורגישות. צריך לחזור על הניסוי בכמות חיידקים גדולה מאוד.

דניאלה שך – לדבר איתה על מיקרוסקופ אלקטרונים

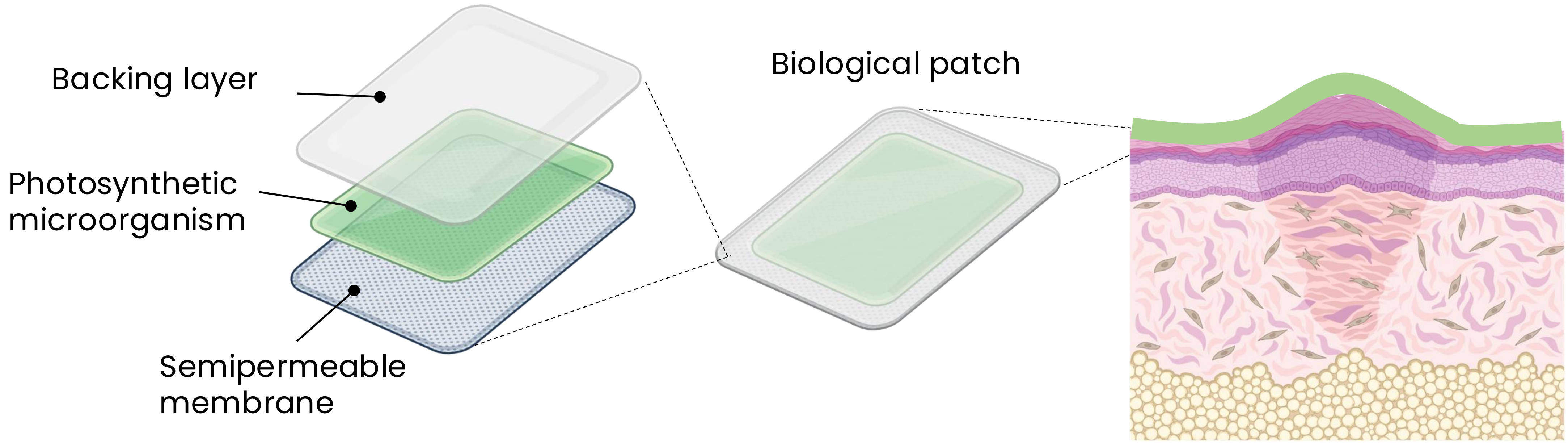
לחזור למאמרים של אסף ורדי וקפלן – תופעה ידועה

אקסודטים של צמחים לעומת של קלאמי ואצות בכלל

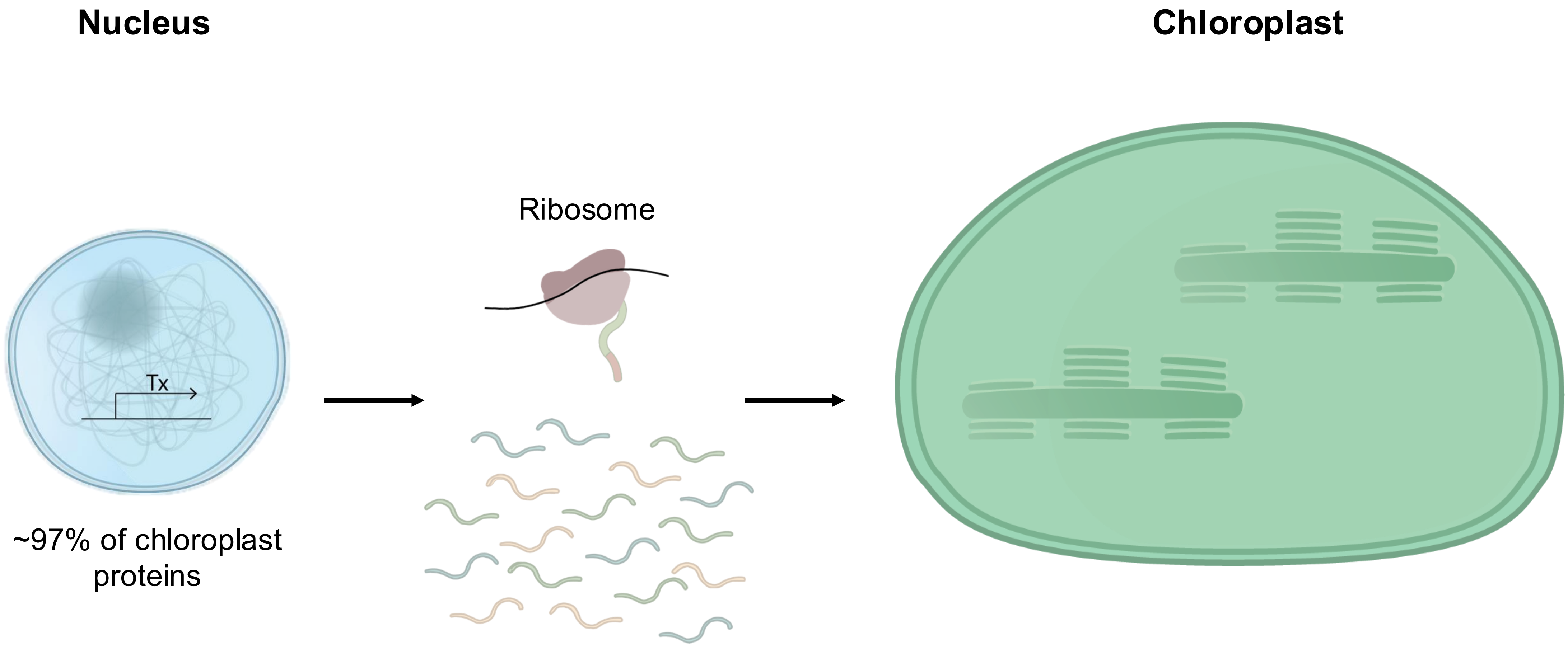
Pyrenoid - density dependent

Eyespot – carotenoid rich, photoreceptors and thylakoids to sense light and swim towards or against

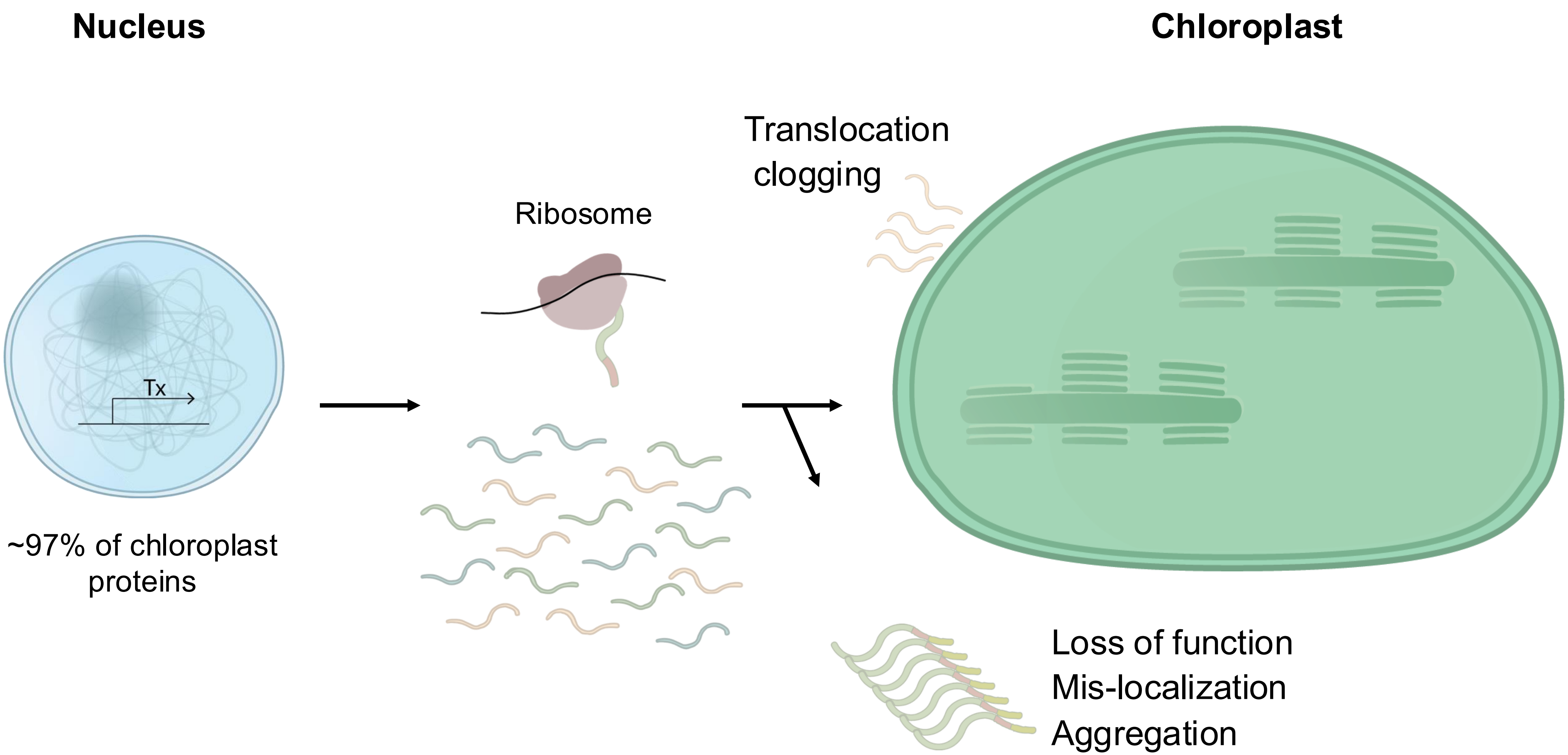
CultivO2 is pursuing a high-impact opportunity to apply its unique microalgae and hydrogel technologies to the rapidly growing field of skin regeneration and chronic wound healing.



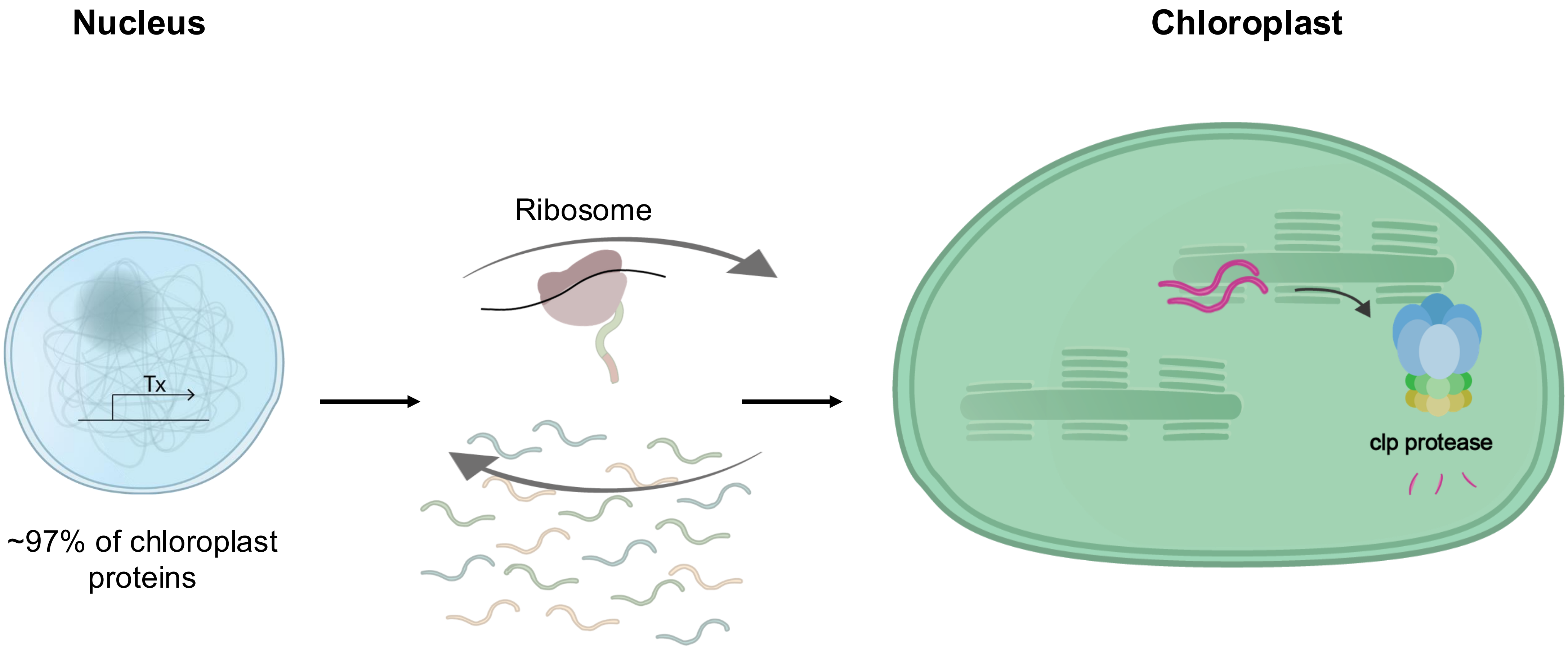
The vast majority of chloroplast resident proteins are encoded by nuclear genes



What dictates the chloroplast proteome composition?

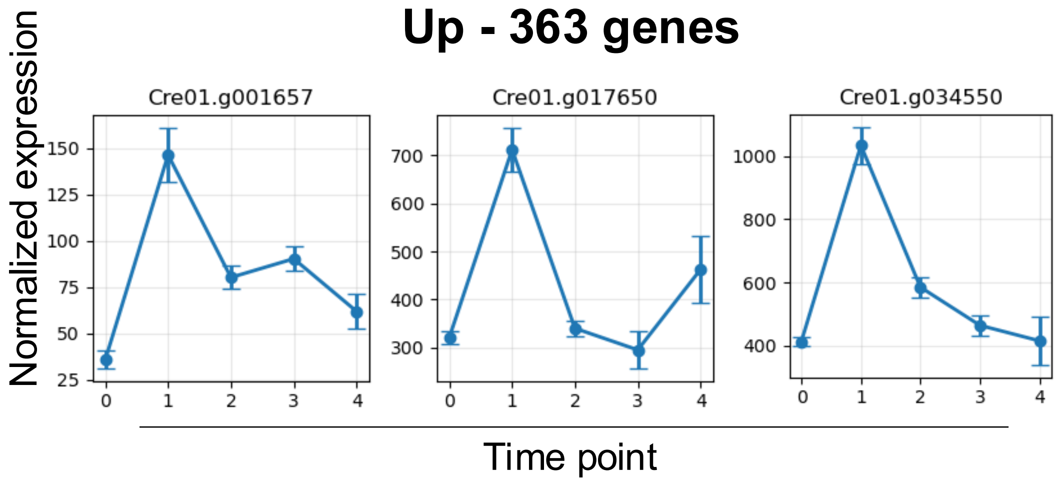


Most of the factors involved in chloroplast protein quality control (PQC) are encoded by the nucleus

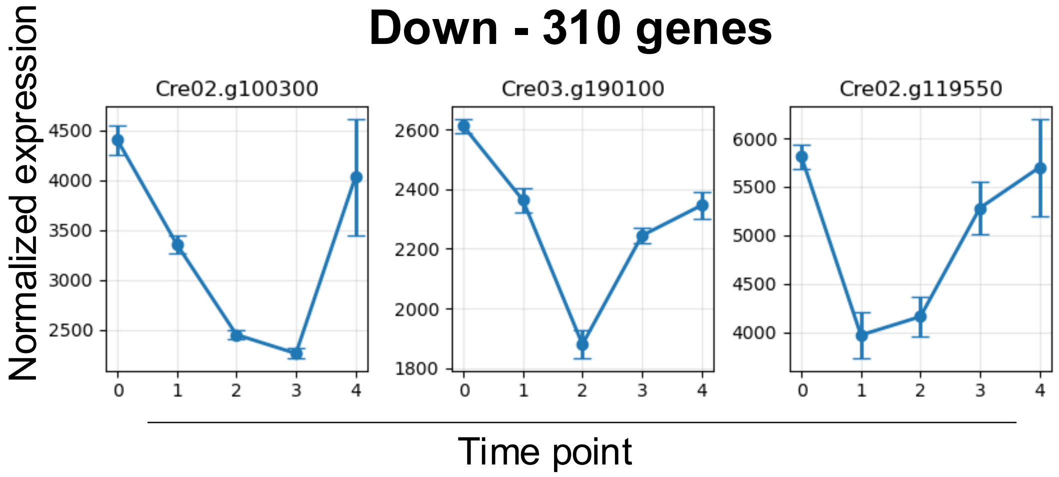


Examining the abrupt response towards the pathogen

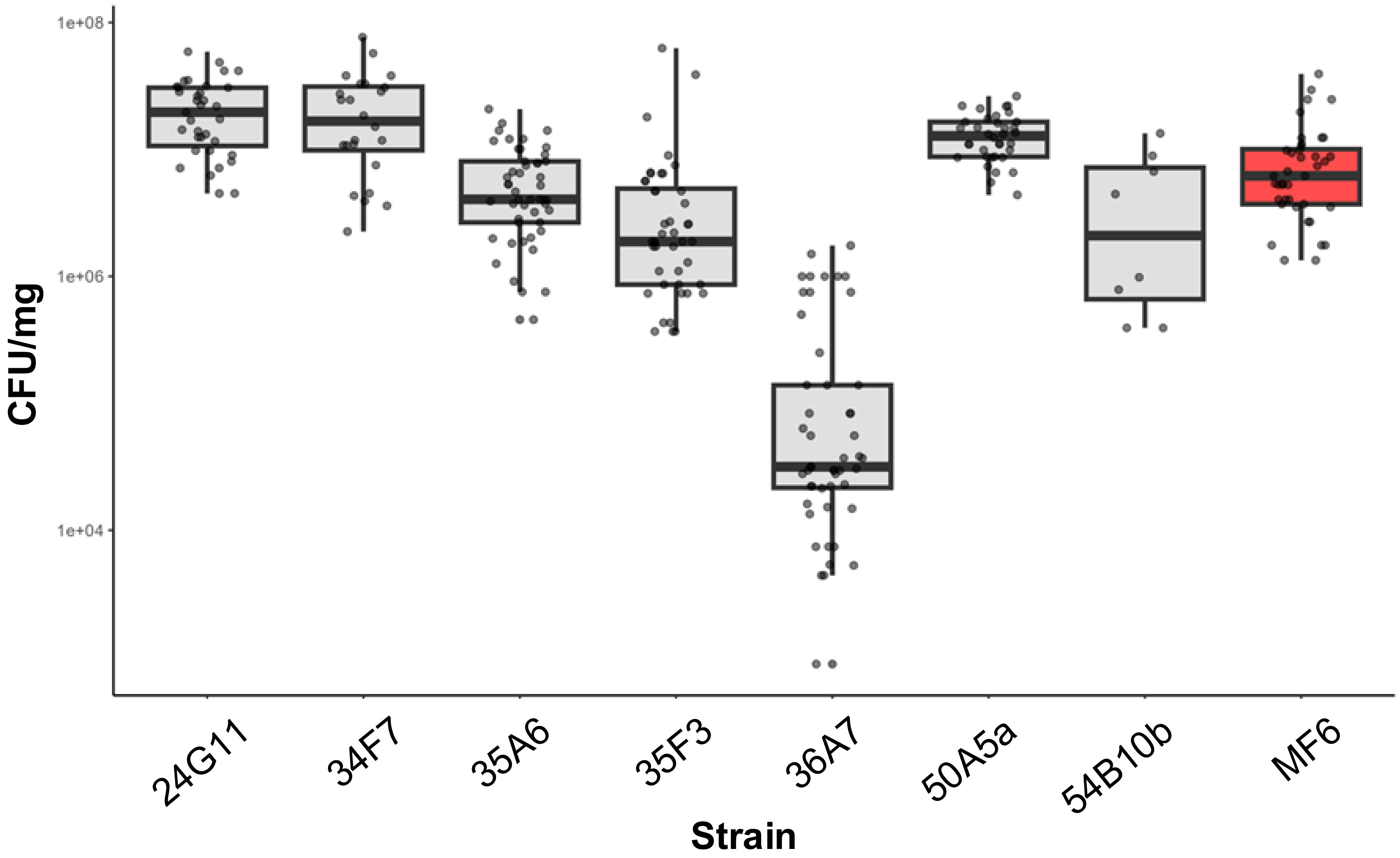
cytoplasmic microtubule organization
microtubule-based movement
flagellum-dependent cell motility
intraciliary transport
protein refolding and polymerization
lipid catabolic process



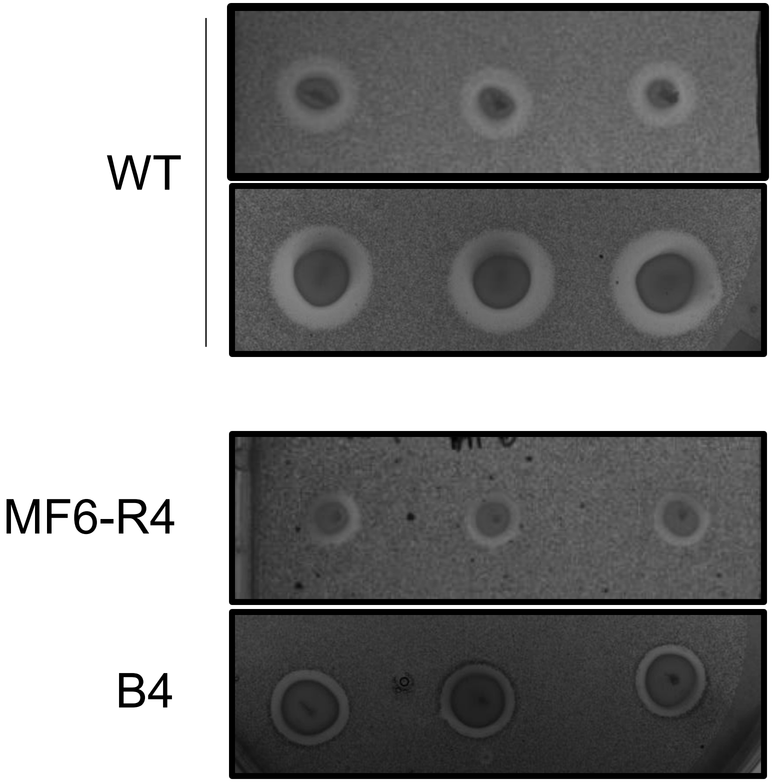
translation initiation
transmembrane transport
metabolic processes

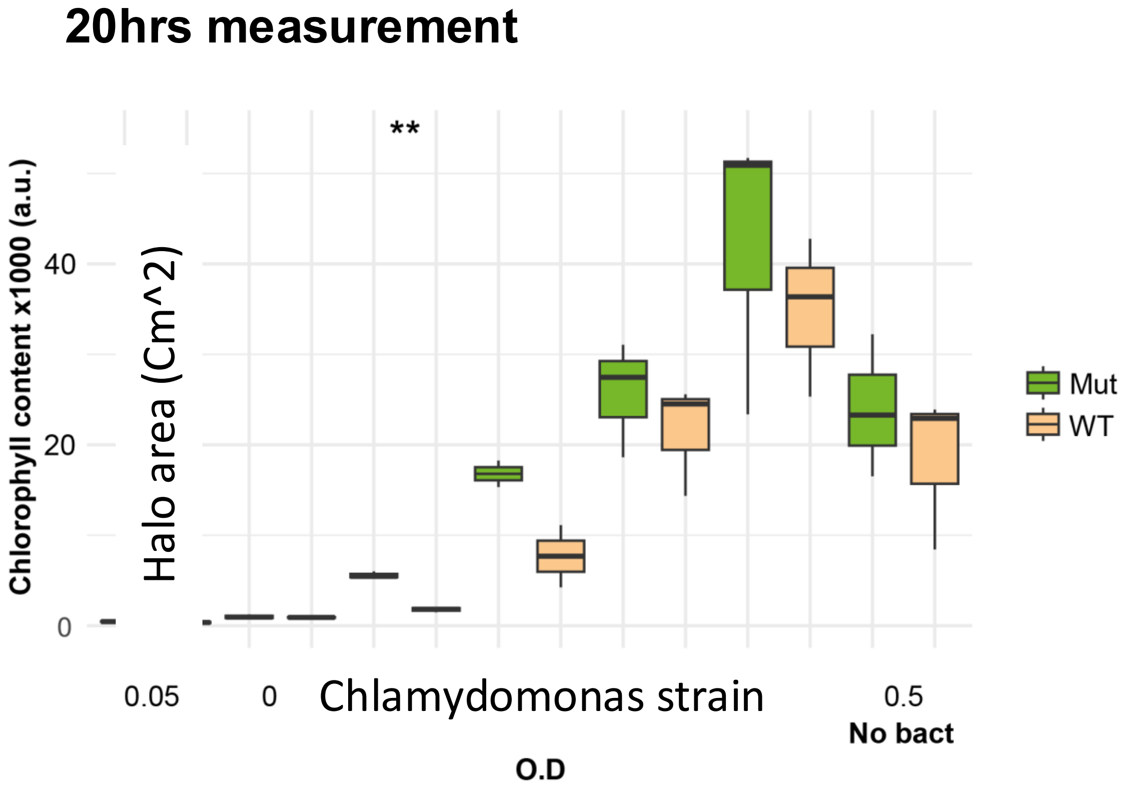


Four out of five mutant strains tested colonize as WT



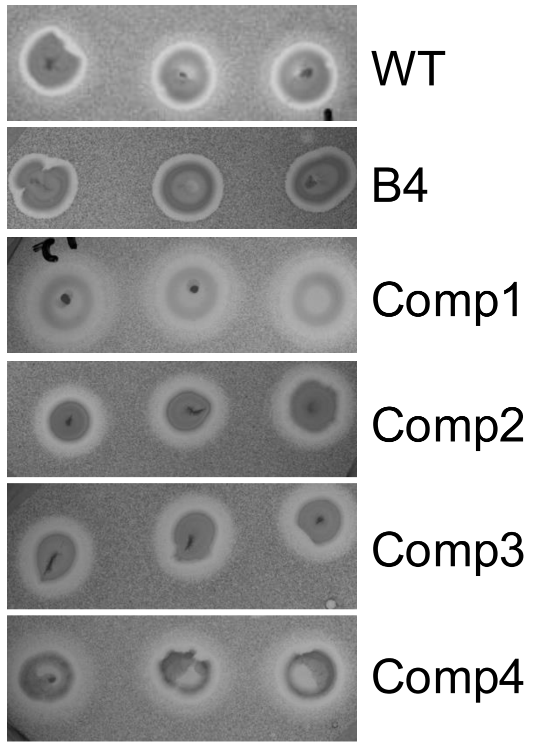
Two resilient mutant strains were isolated



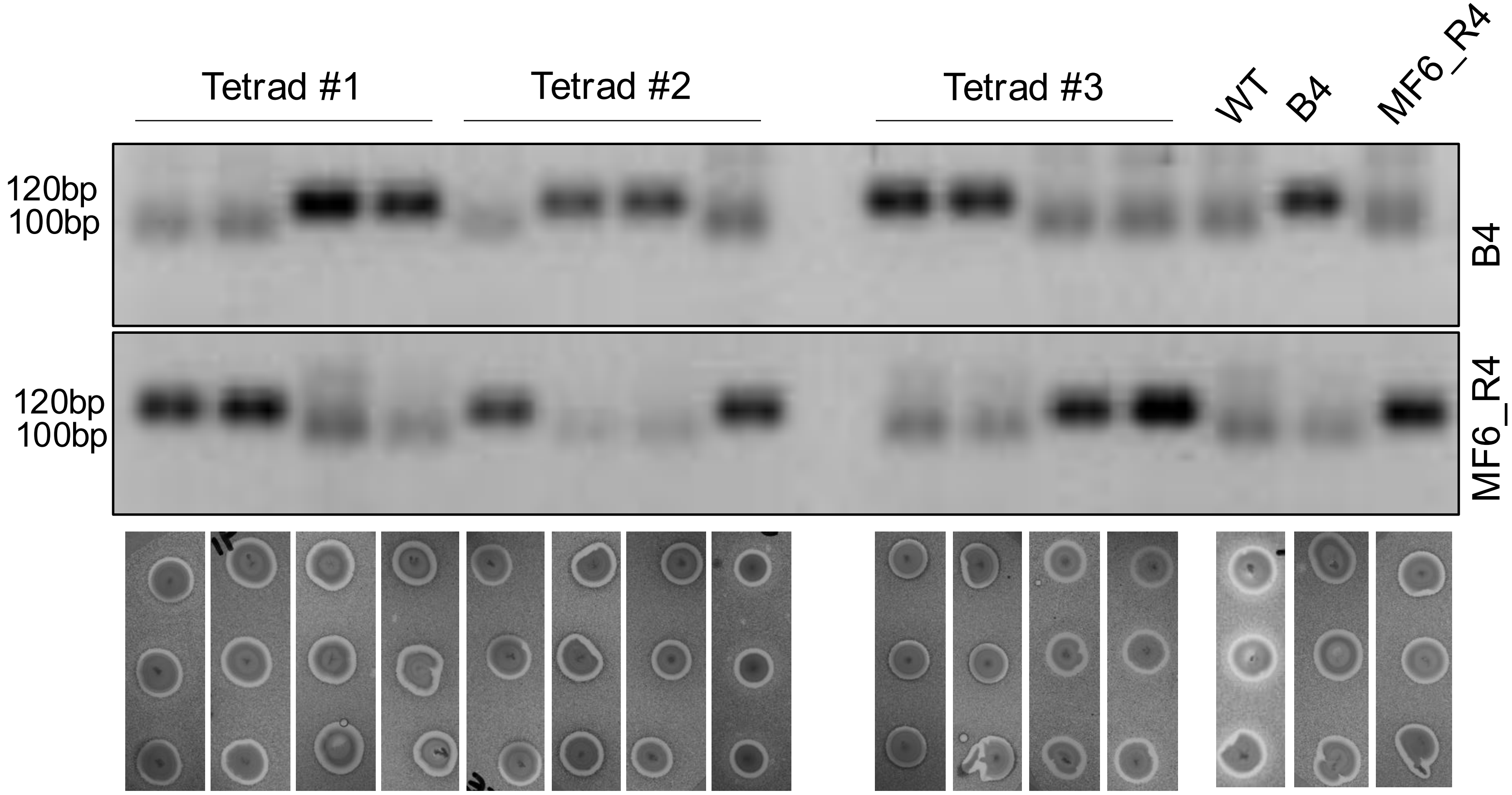


Causality was verified by complementation and linkage

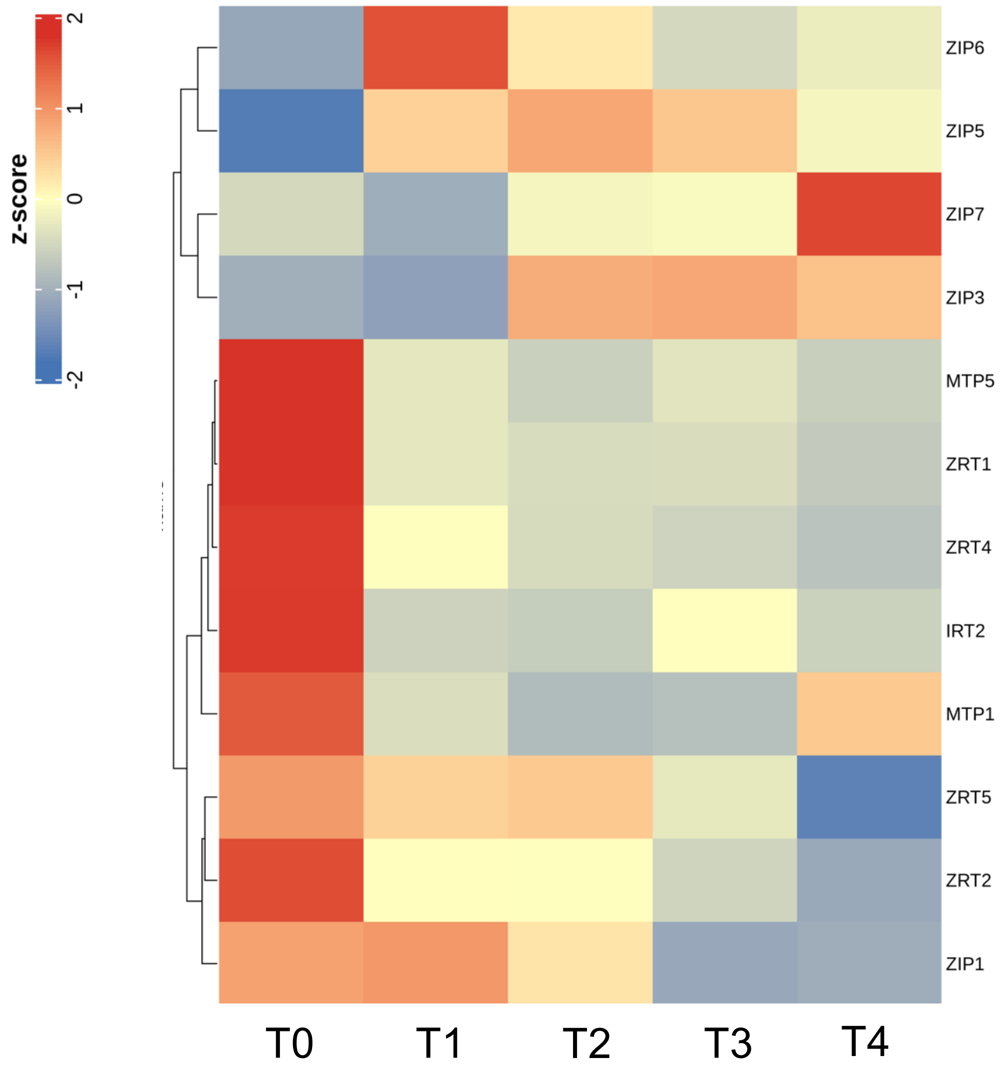
Complementation

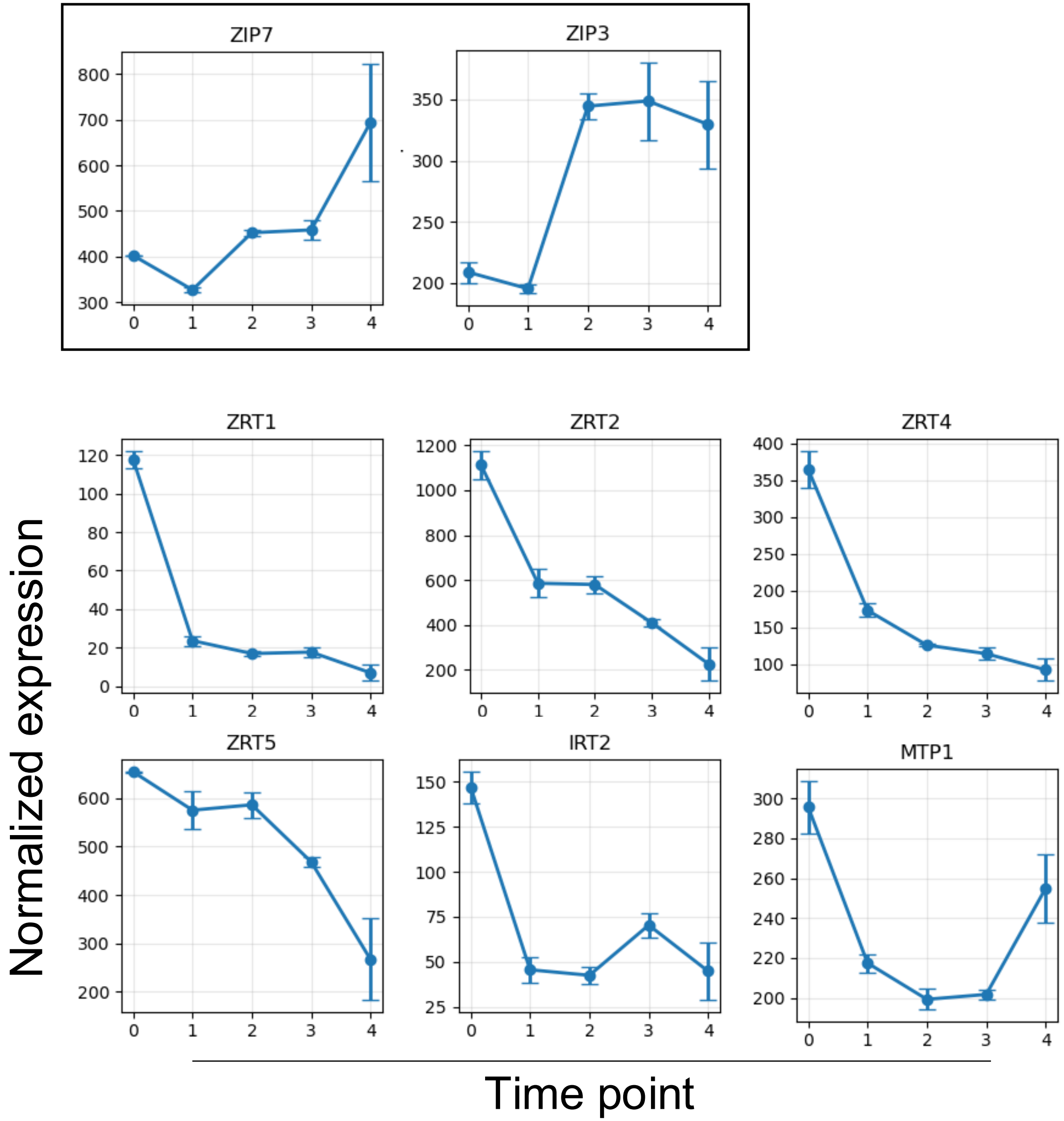


Genetic linkage

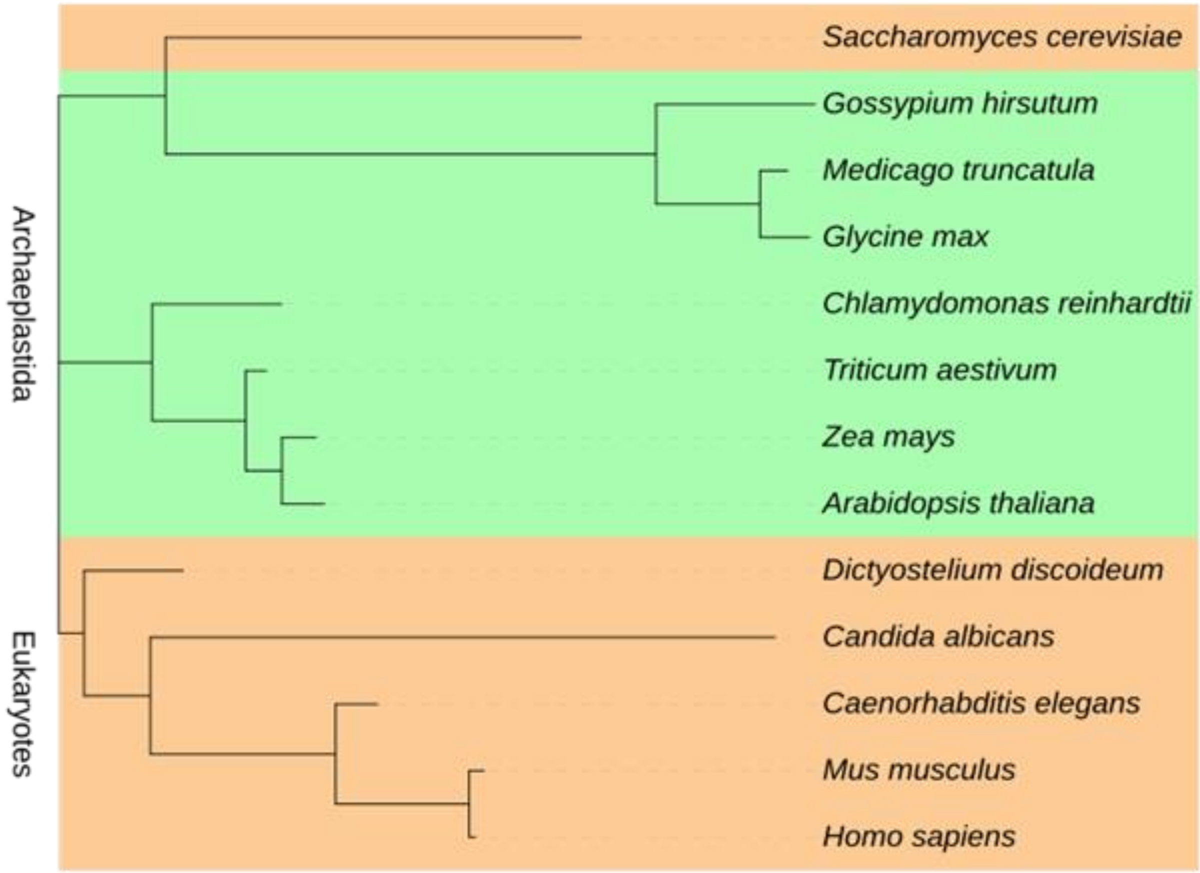


ZIP3 is significantly upregulated

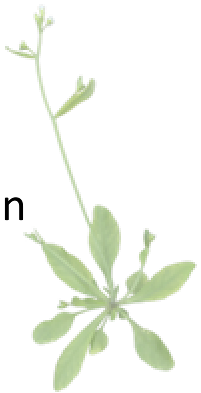




The closest homolog in *Arabidopsis* is ZTP29 (AT3G20870)



ZTP29 homolog in Arabidopsis
Transmembrane protein located in the ER.



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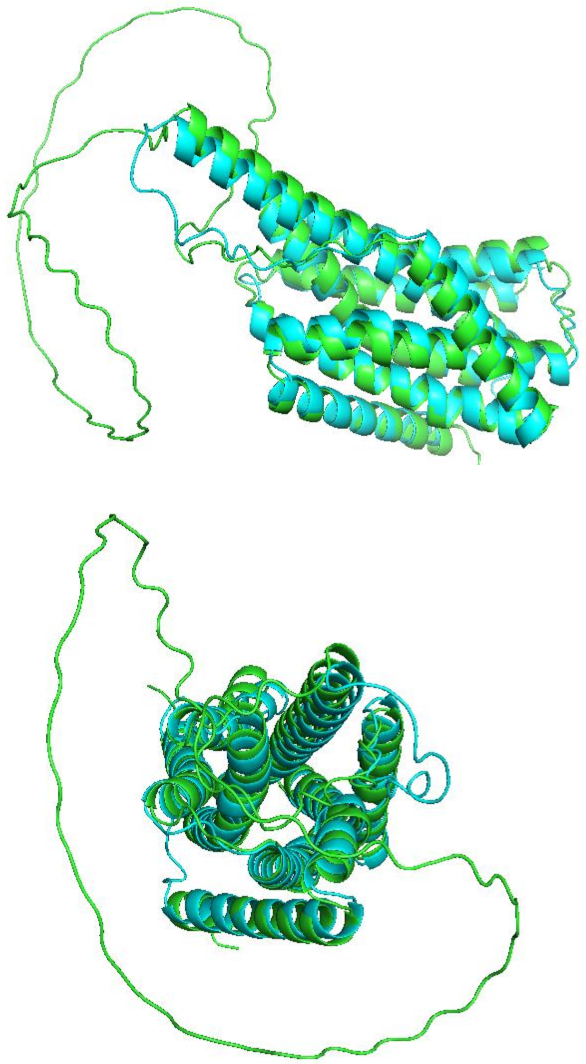
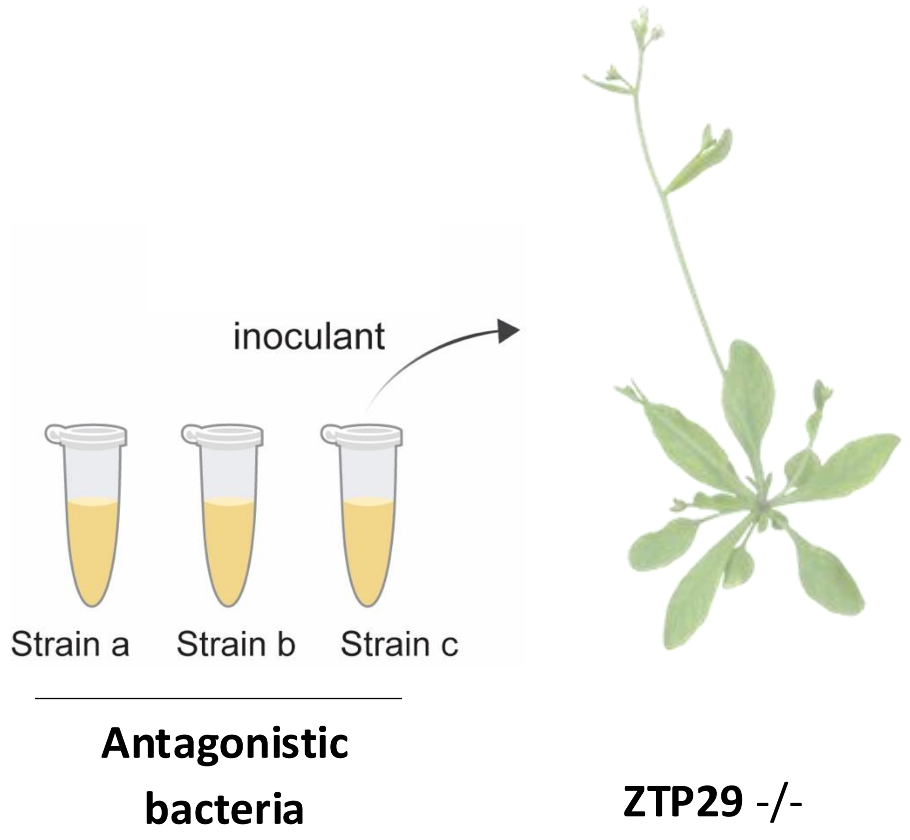


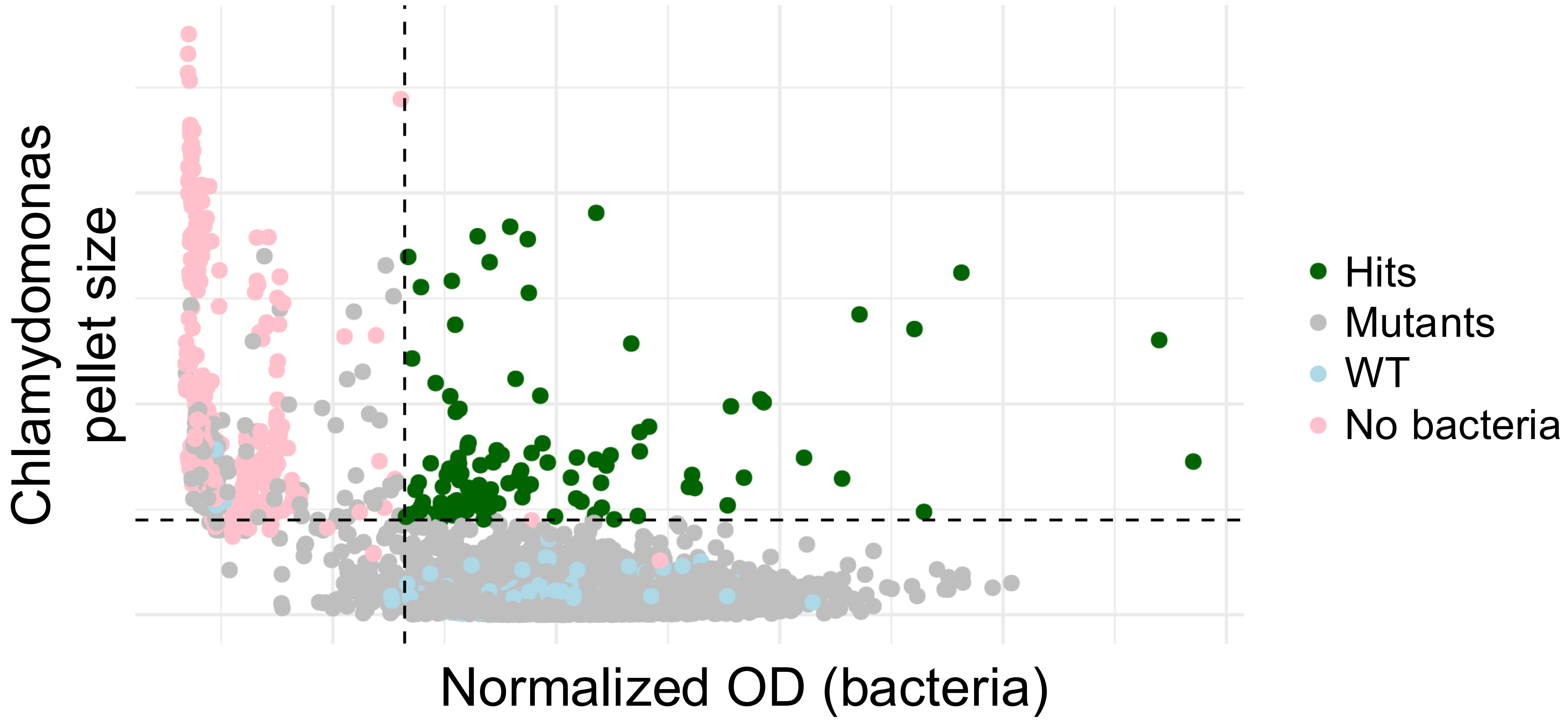
Green: ZIP3
Blue: AT3G20870

RMSD(align)=1.045

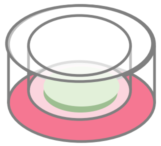
Identity and similarity 58%

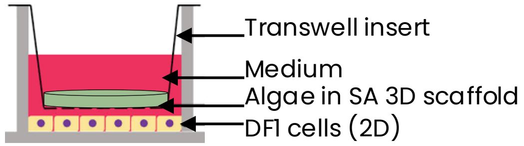


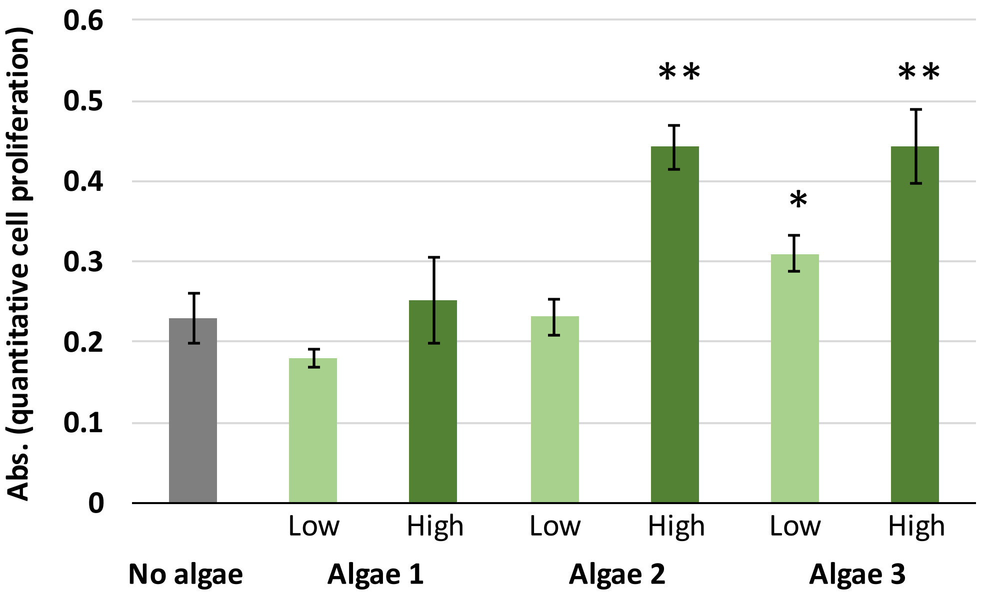
Multiple MF6 mutants lost their virulence



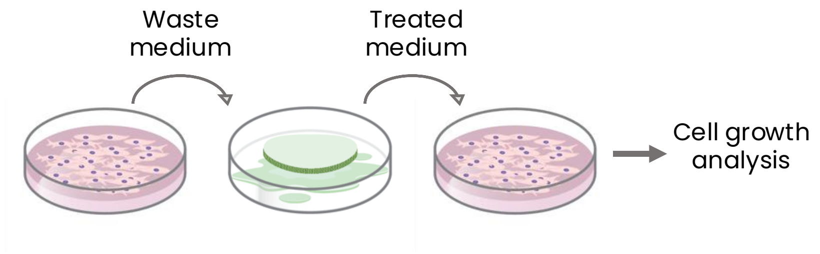
Microalgae Presence Doubles the Biomass Within Two Days

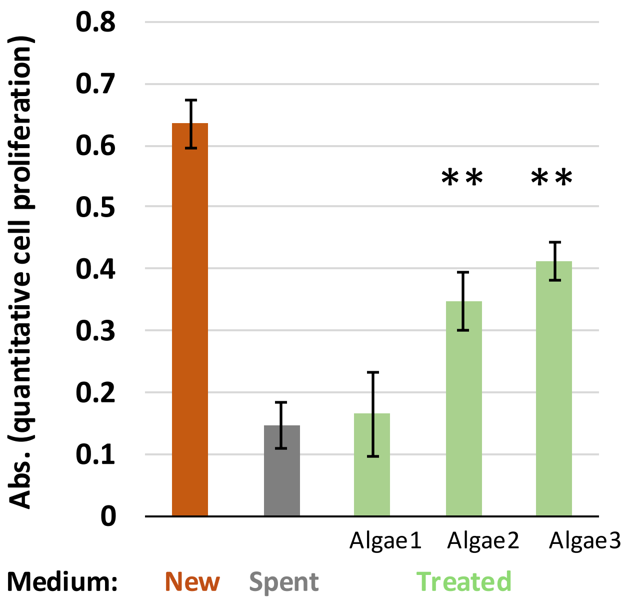


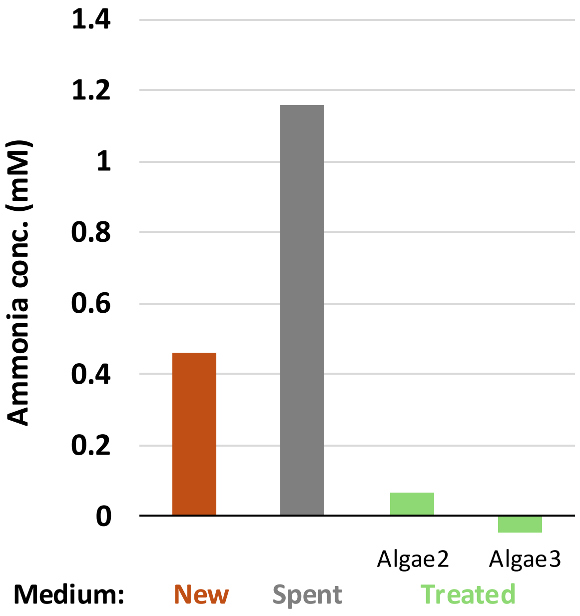




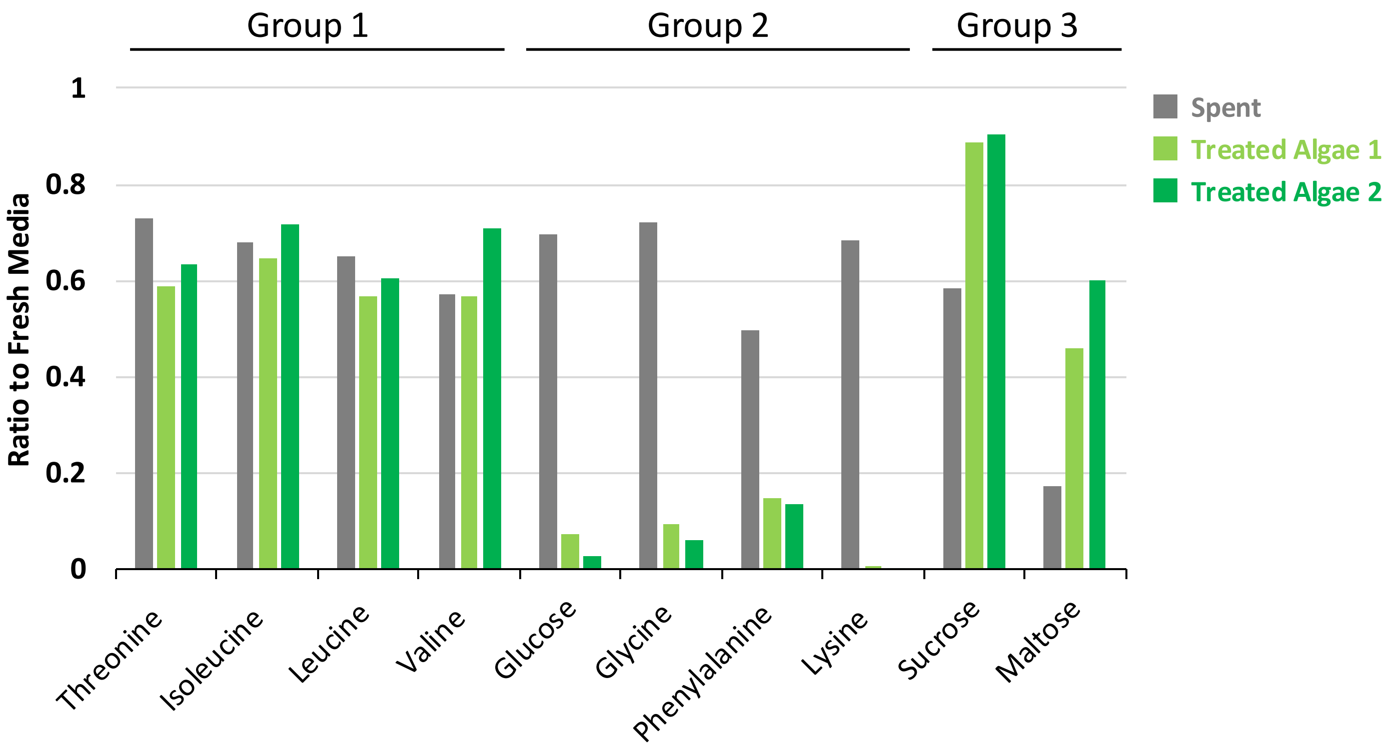
Microalgae Restore Spent Medium and Consume Toxic By-Products







Gas Chromatography/Mass Spectrometry (GC-MS) Analysis of Spent and Algae Treated Media

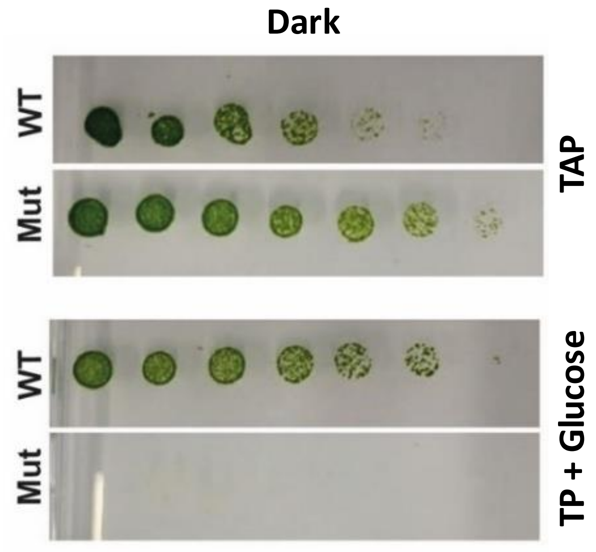


- Group 1- consumed by animal cells
- Group 2- consumed by both animal cells and microalgae
- Group 3- consumed by animal cells replenished by microalgae

The goal is to optimize symbiosis by enhancing photosynthesis and toxic byproduct consumption while minimizing nutrient competition.

Genetically Modified Microalgae for Optimized Symbiosis

Glucose consumption by microalgae not only compete with animal cells on essential nutrients but also **reduces photosynthesis rates**



We developed microalgae that doesn't require external glucose supplementation and can effectively carry out photosynthesis.

We developed microalgae that produce the growth factor bFGF (data not shown).