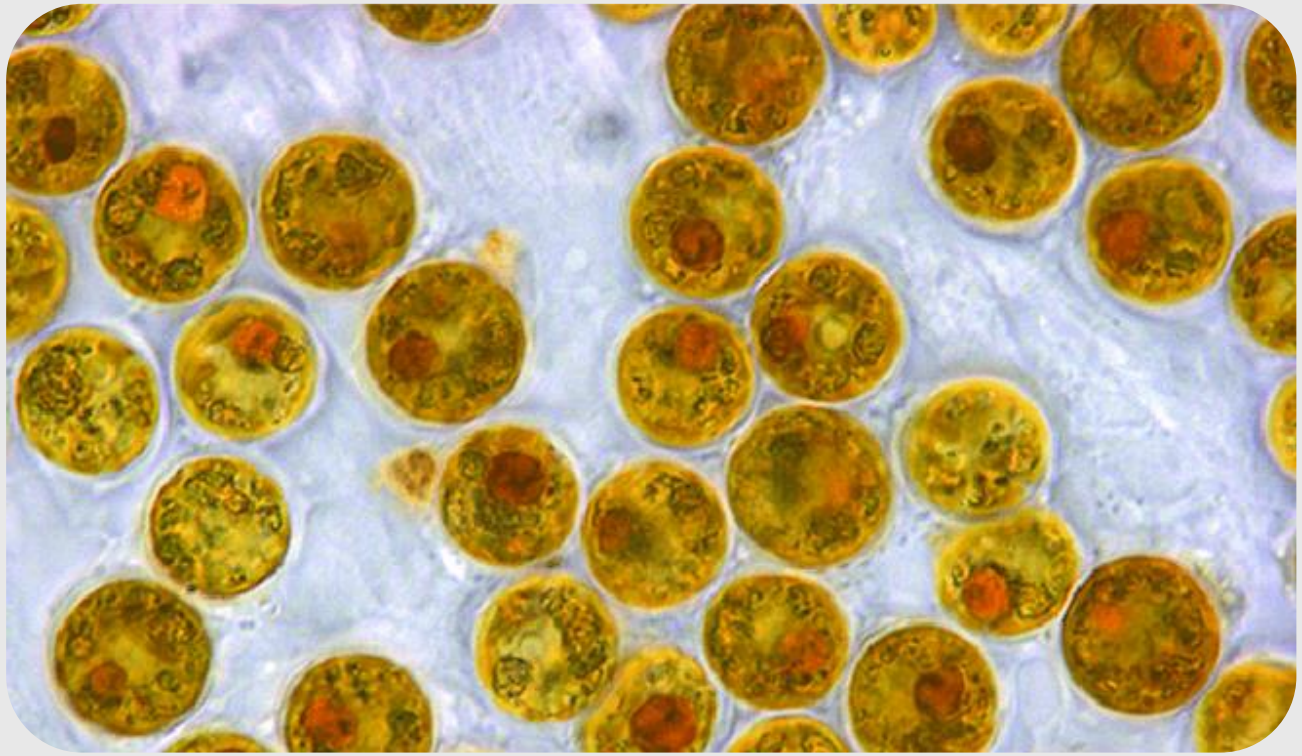


Analysis of ***Symbiodinium microadriaticum*** **mutant** strains with impaired PSII activity

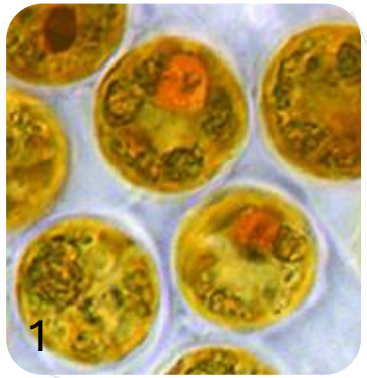
University of Liège
InBios, Phytosystems

Second European Congress on Photosynthesis Research (ePS2)

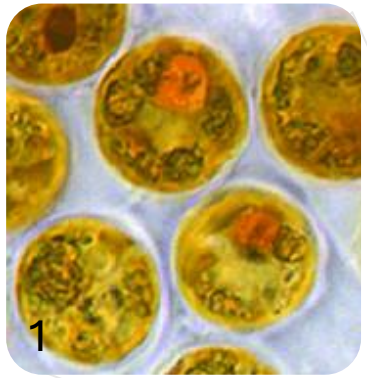
26.06.24

Edmée Royen

Introduction – Symbiodiniaceae



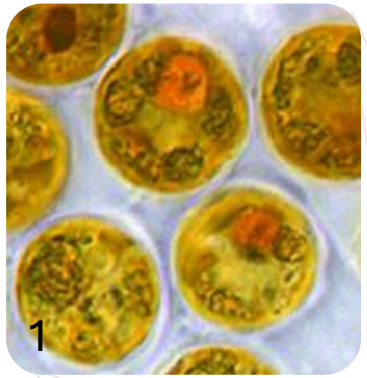
1. **Introduction** – Symbiodiniaceae



Photosynthesis products

Stability, Inorganic compounds

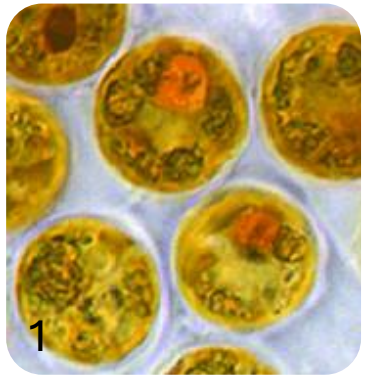
1. **Introduction** – Symbiodiniaceae

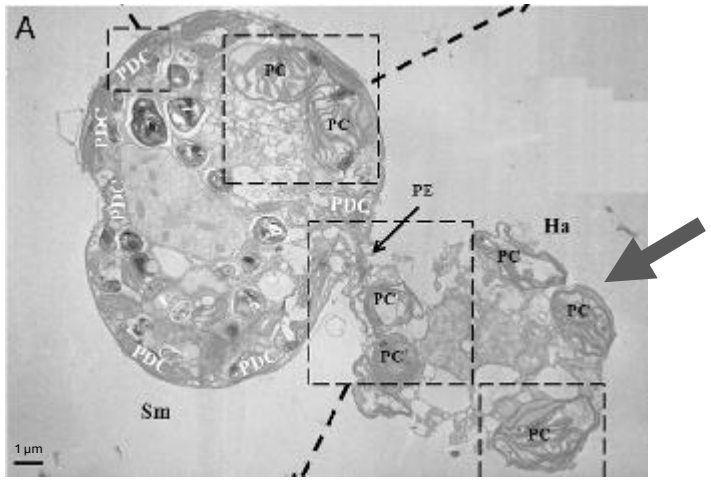


Photosynthesis products

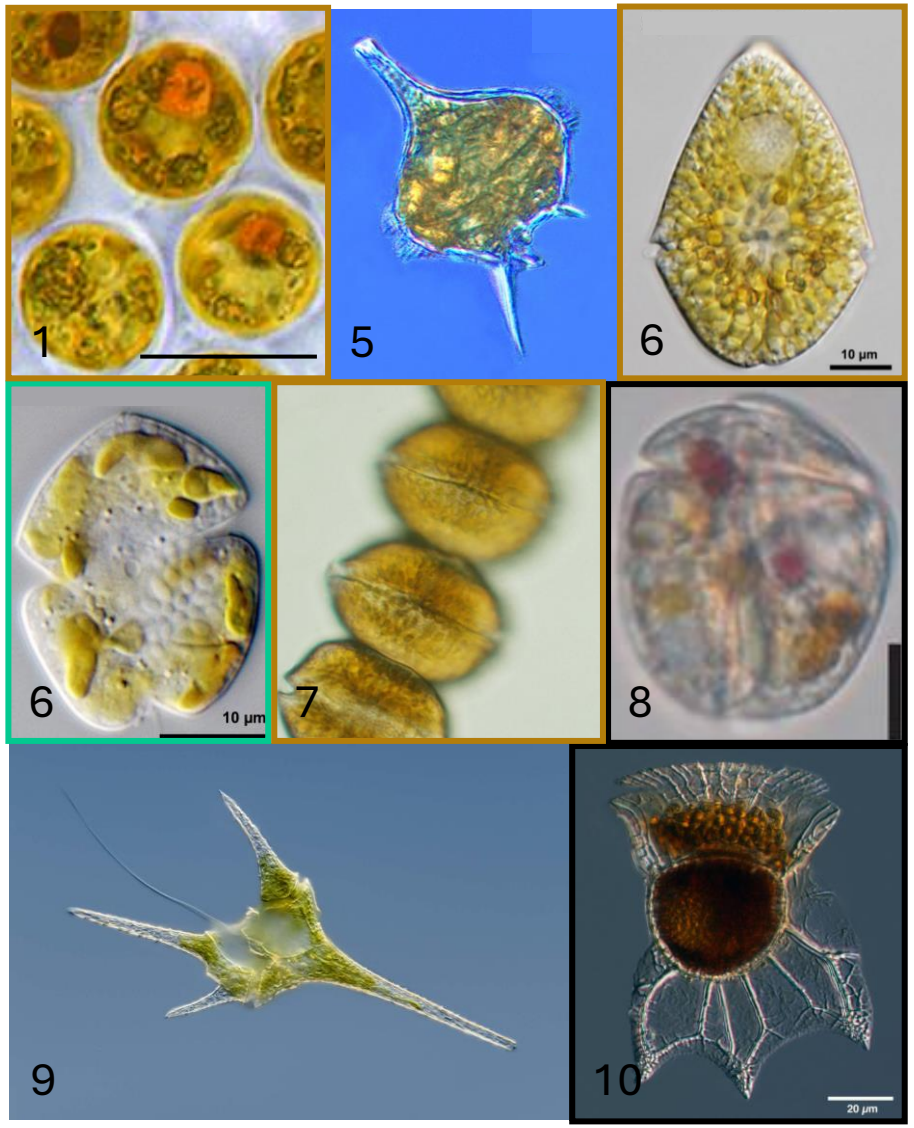
Stability, Inorganic compounds

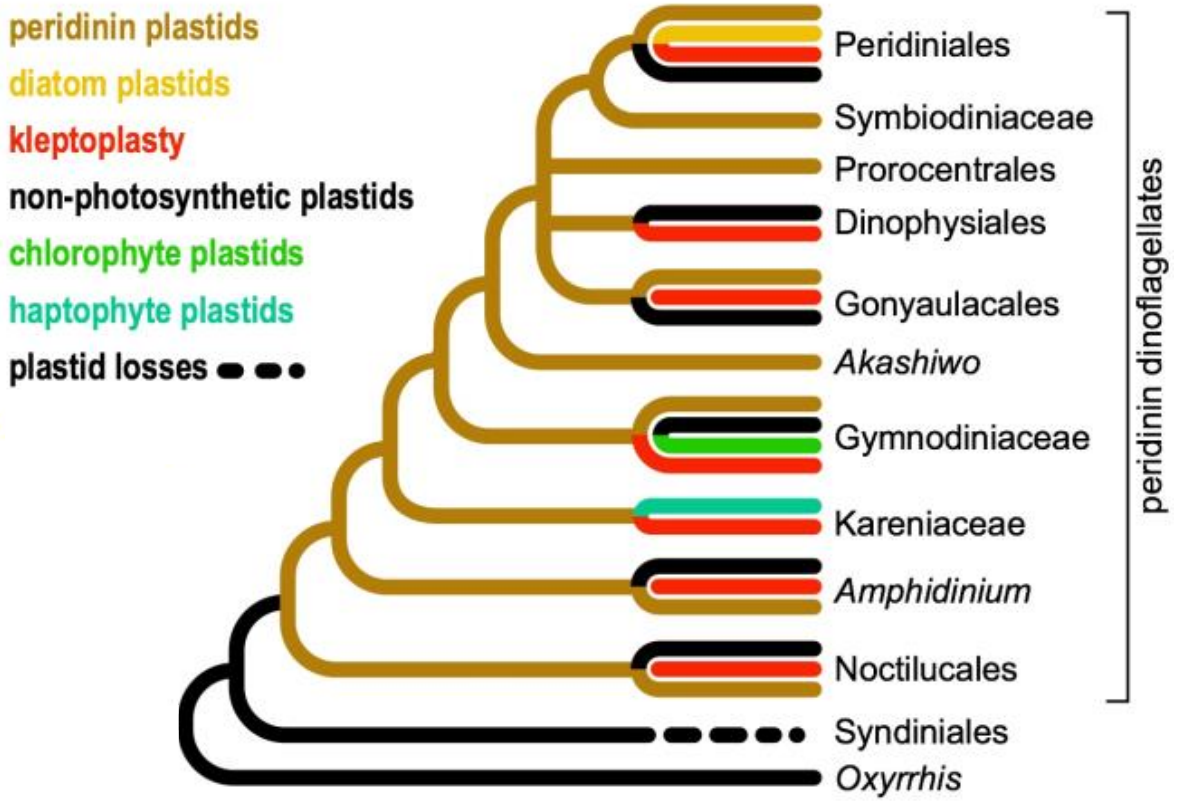
1. **Introduction** – Symbiodiniaceae



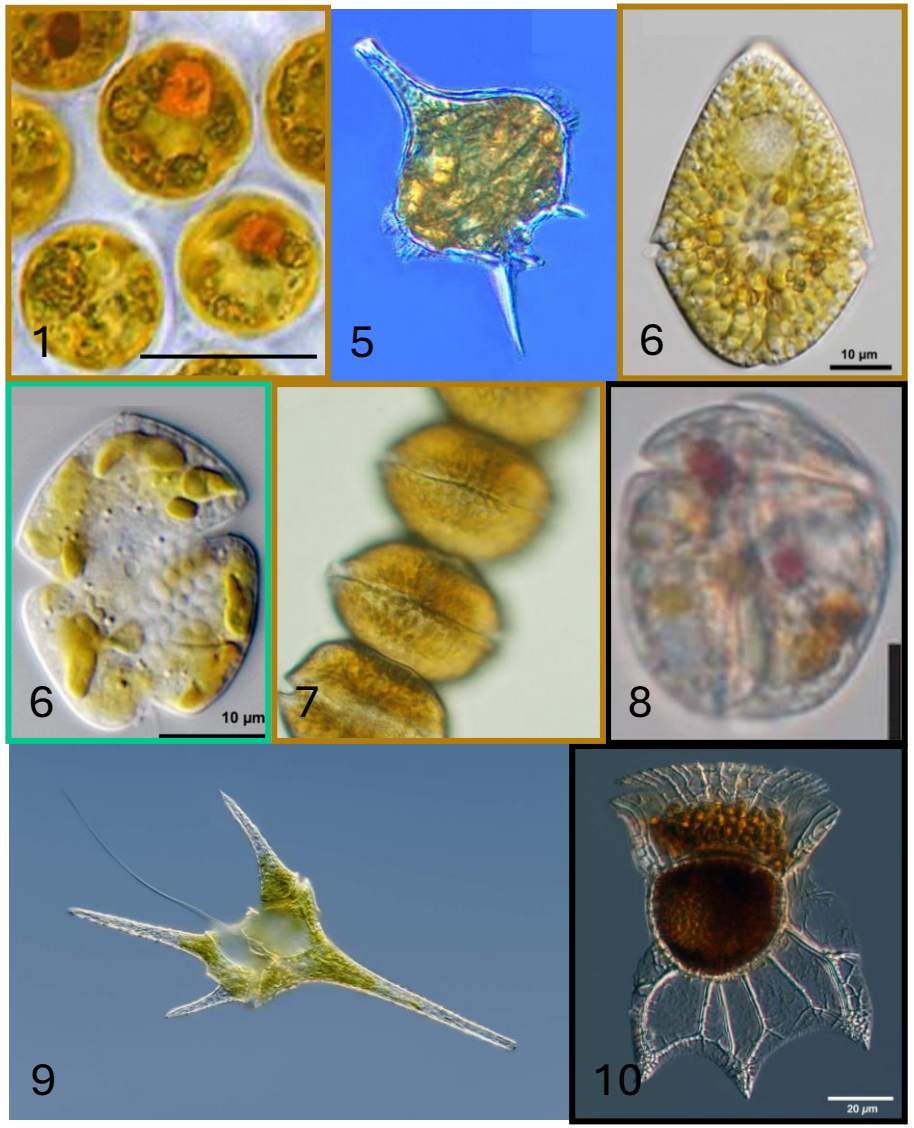


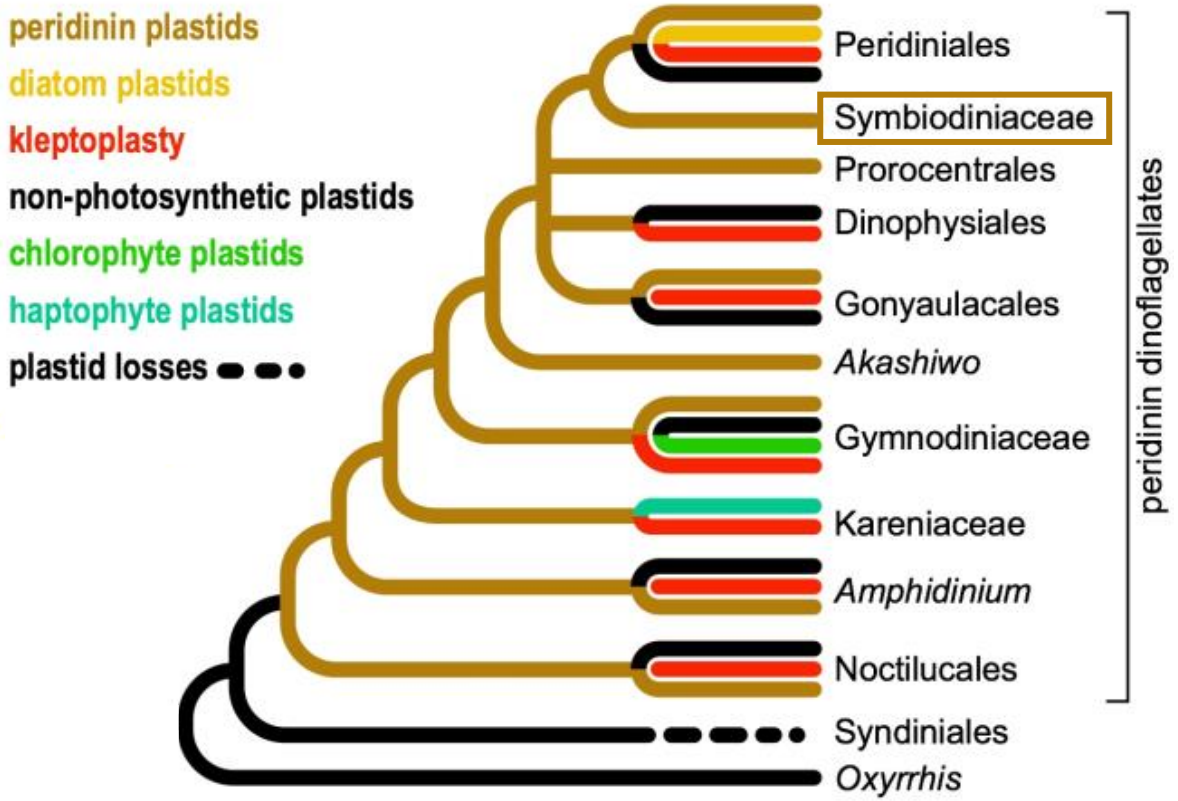
Jeong 2012 (4)





Adapted from Waller 2017 (6)





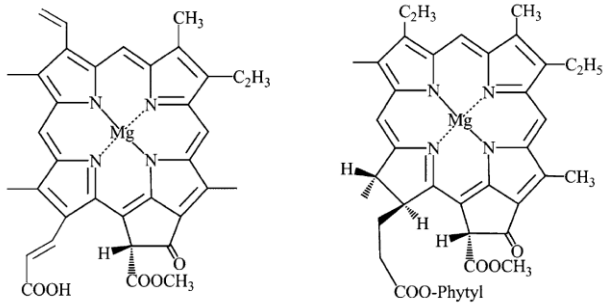
Adapted from Waller 2017 (6)

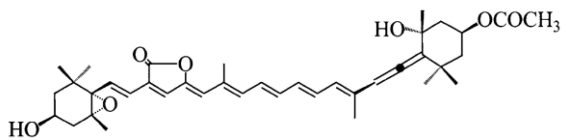
1. **Introduction** – Peridinin plastid

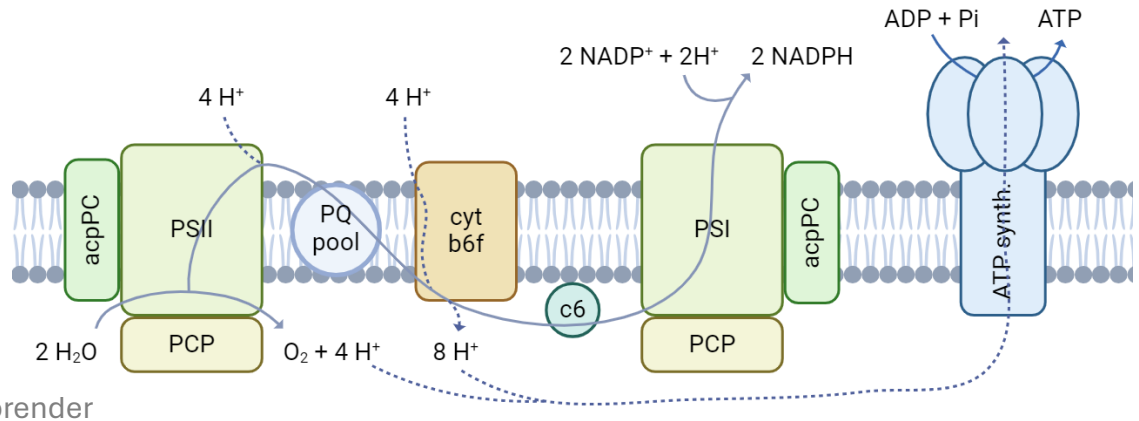
- Type II RuBisCO
- Delimited by 3 membranes

1. **Introduction** – Peridinin plastid

- Type II RuBisCO
- Delimited by 3 membranes
- Main photosynthetic pigments:
  - Chlorophyll a
  - Chlorophyll c2
  - **Peridinin**



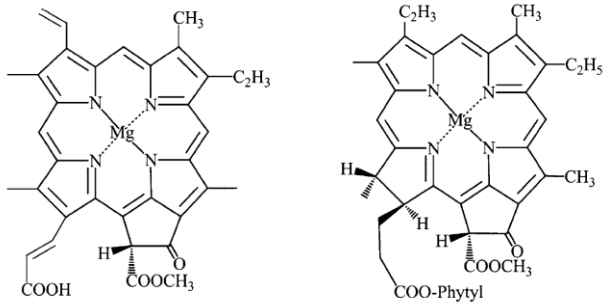


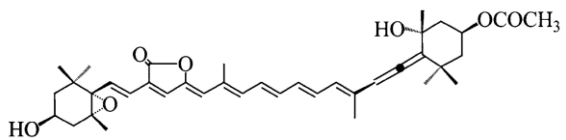


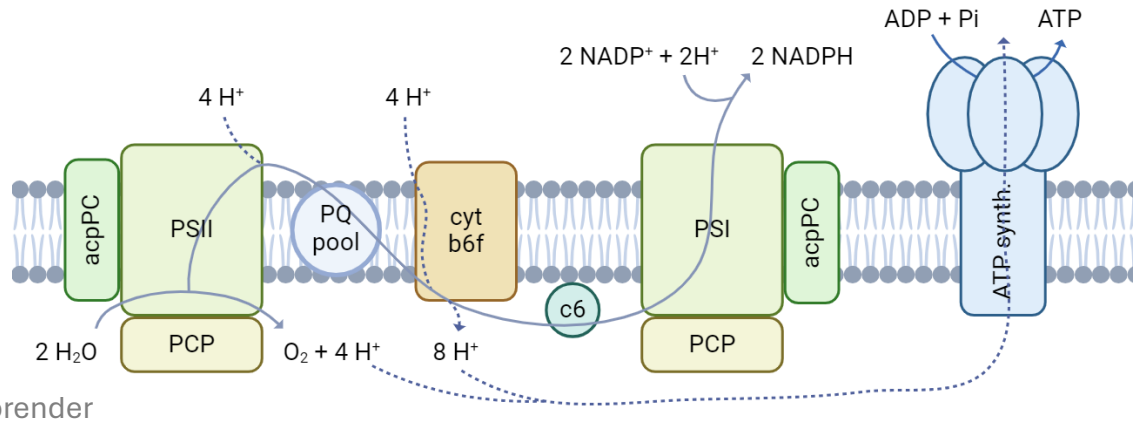
Biorender

1. **Introduction** – Peridinin plastid

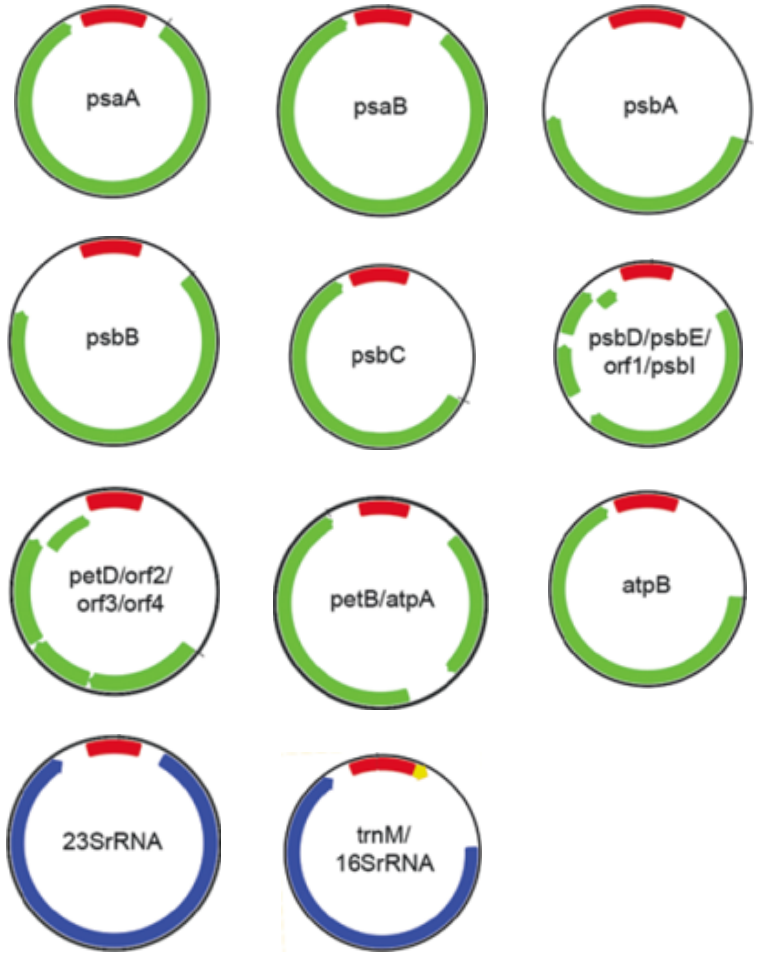
- Type II RuBisCO
- Delimited by 3 membranes
- Main photosynthetic pigments:
  - Chlorophyll a
  - Chlorophyll c2
  - **Peridinin**
- Genome organization
  - 2-3 kpb **minicircles**





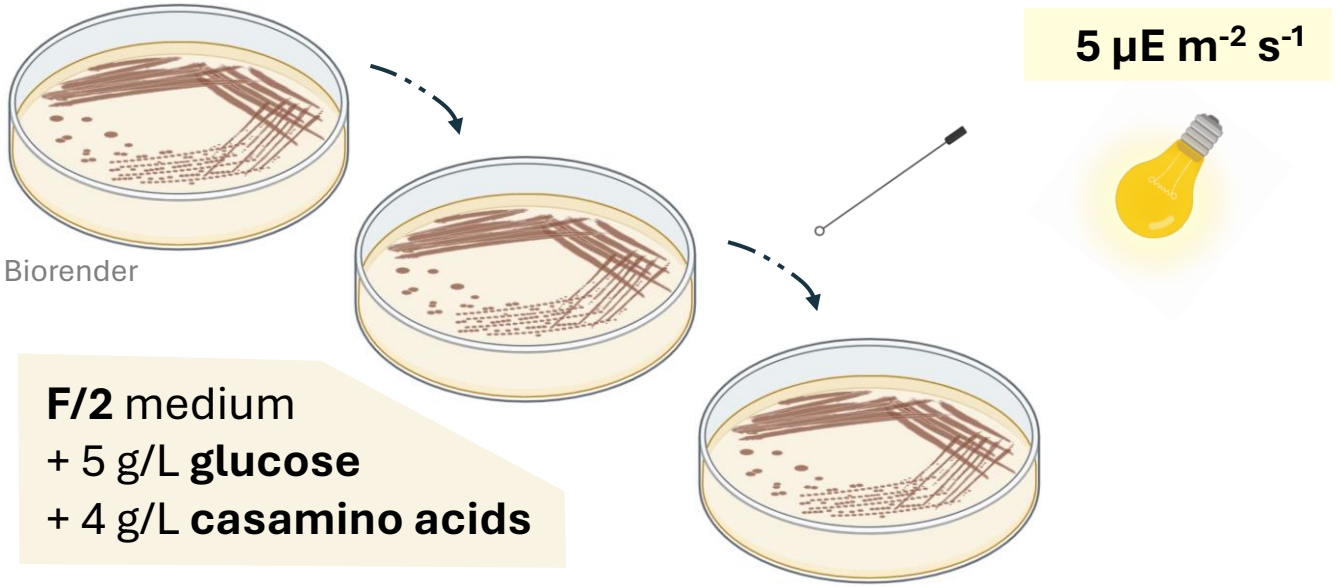


Biorender



from *Amphidinium carterae*
Barbrook 2018 (12)

Adrian Barbrook, Ellen Nisbet, Christopher Howe

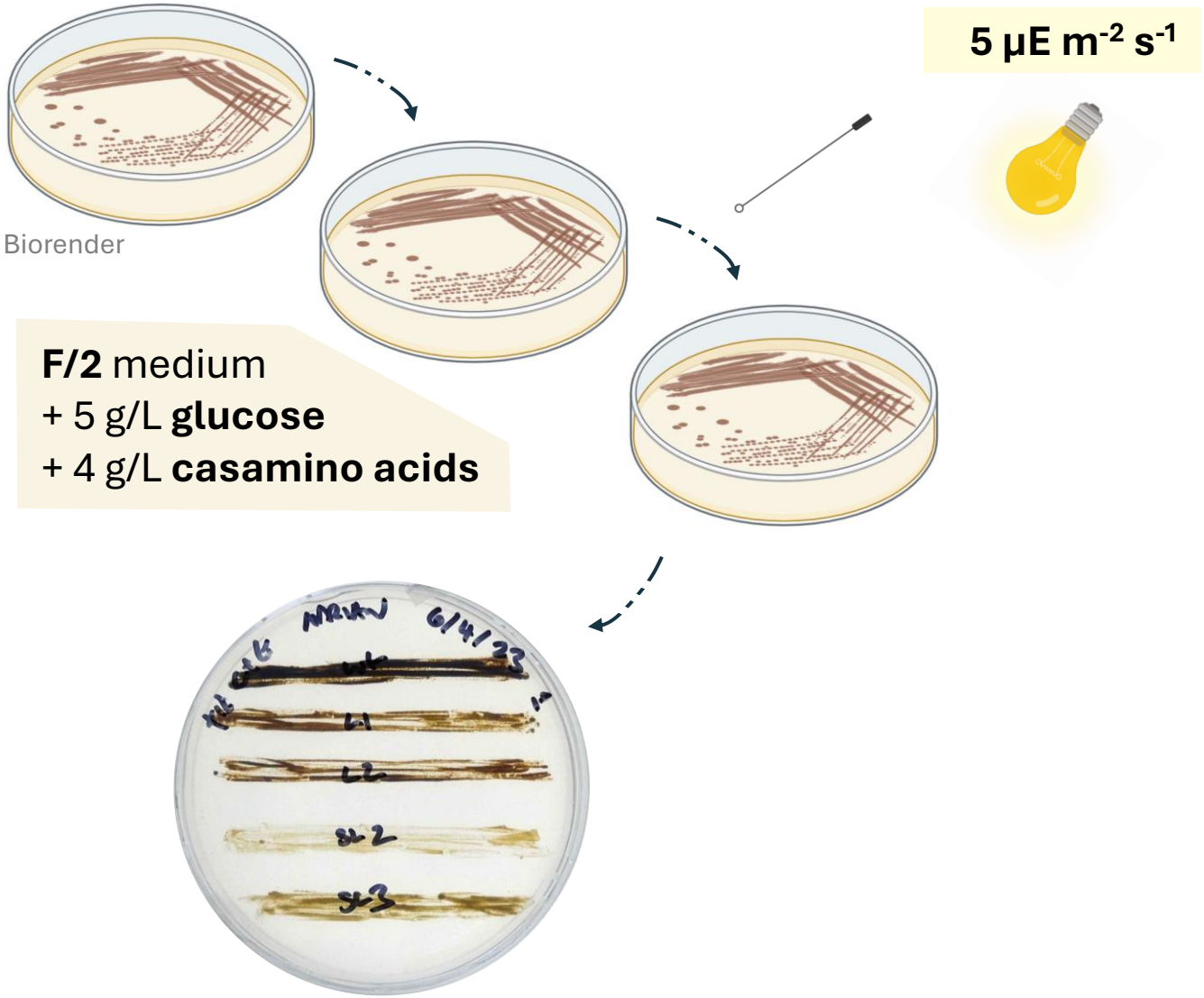


Biorender

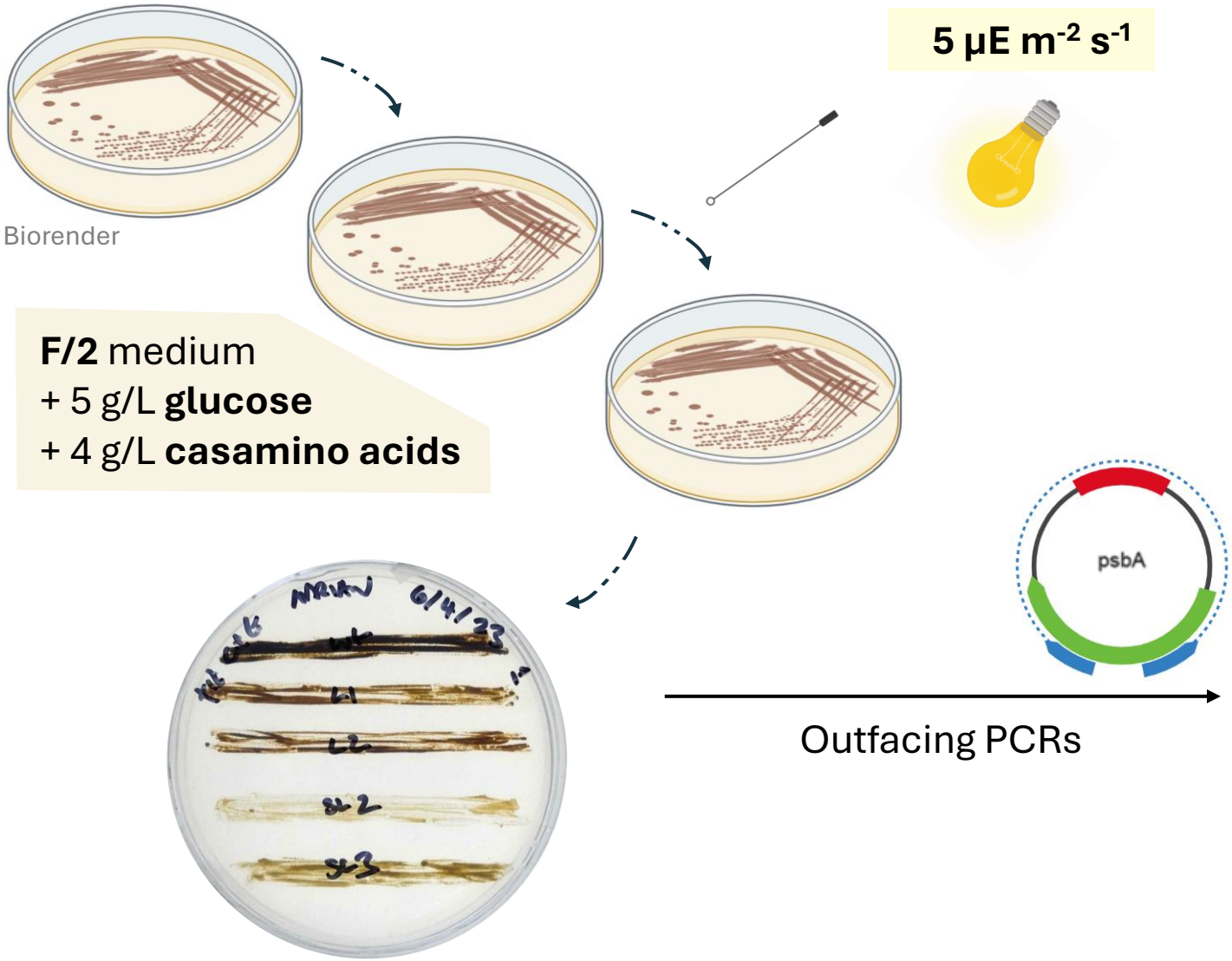
5 µE m^{-2} s^{-1}

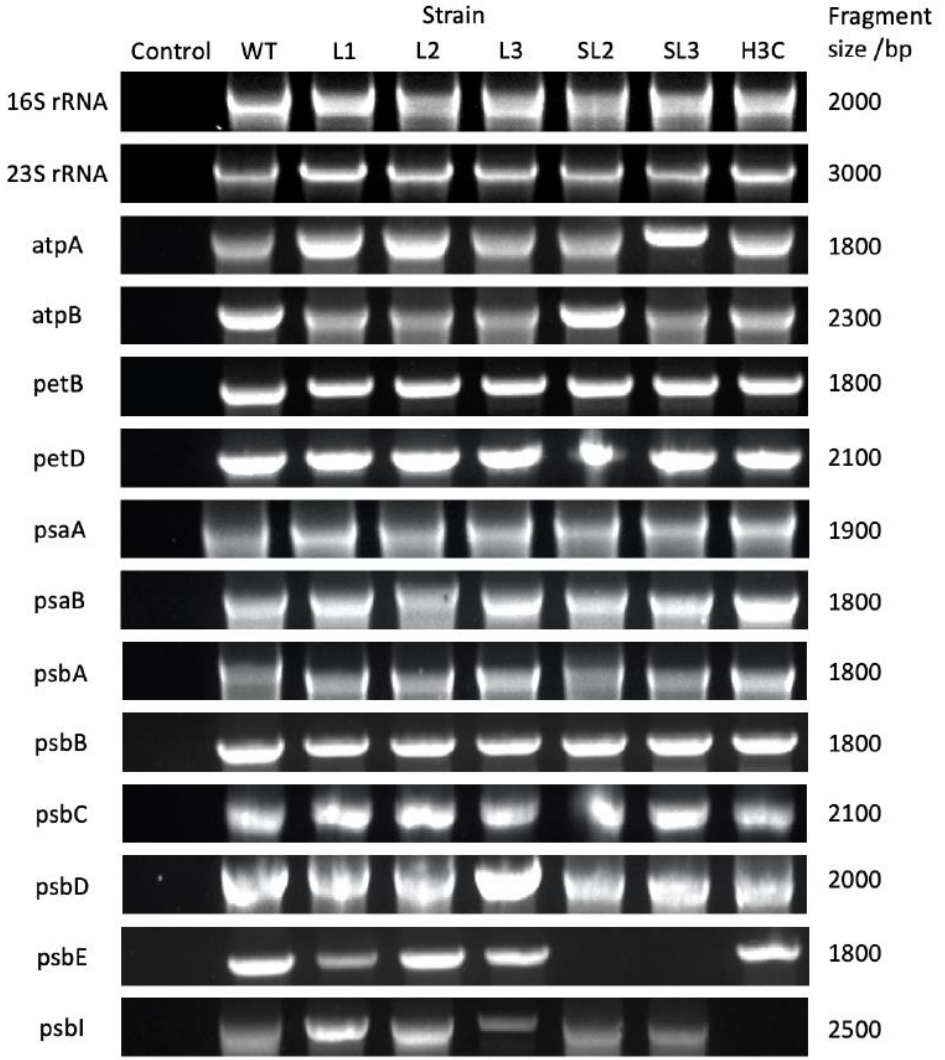
F/2 medium
+ 5 g/L **glucose**
+ 4 g/L **casamino acids**

Adrian Barbrook, Ellen Nisbet, Christopher Howe



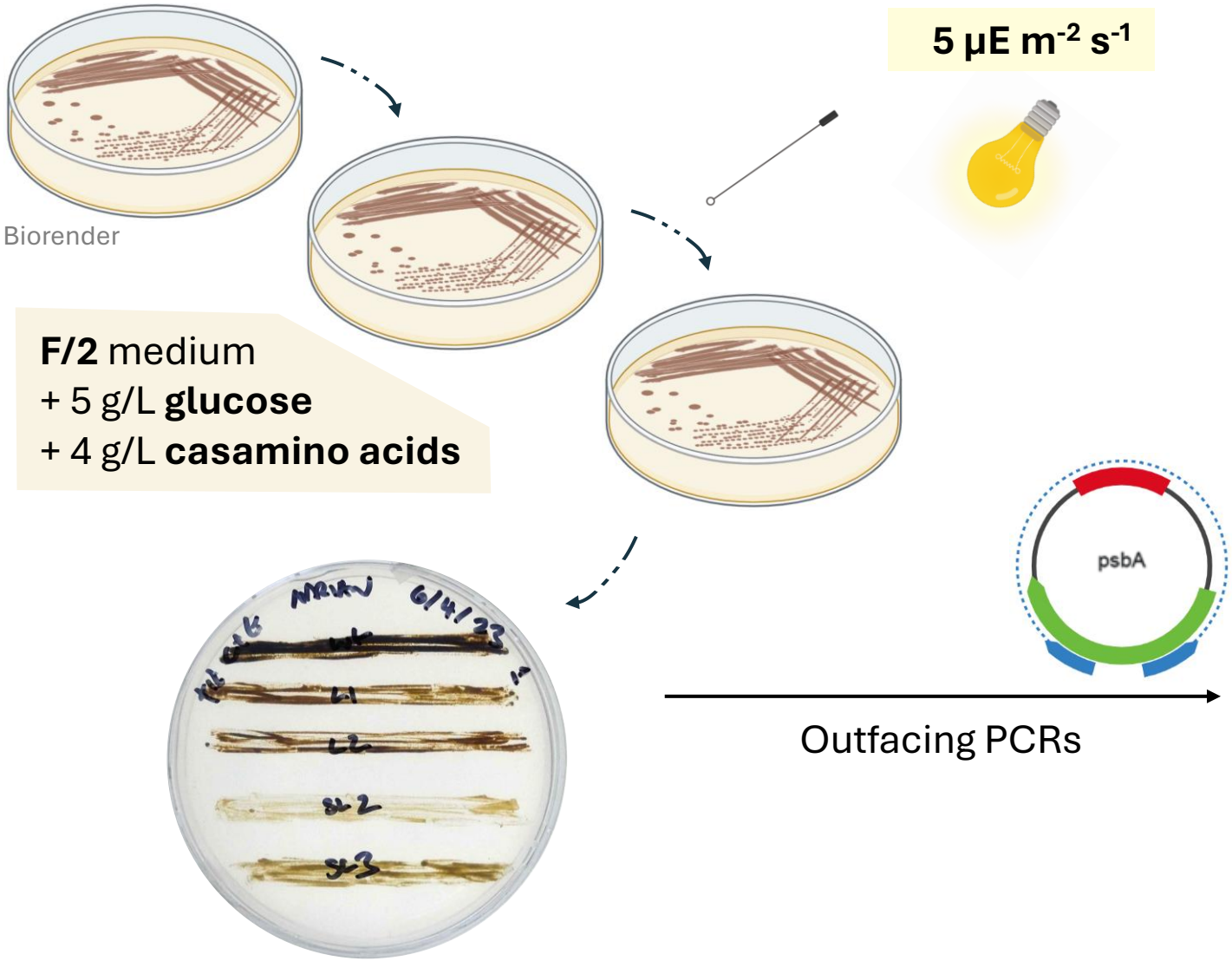
Adrian Barbrook, Ellen Nisbet, Christopher Howe

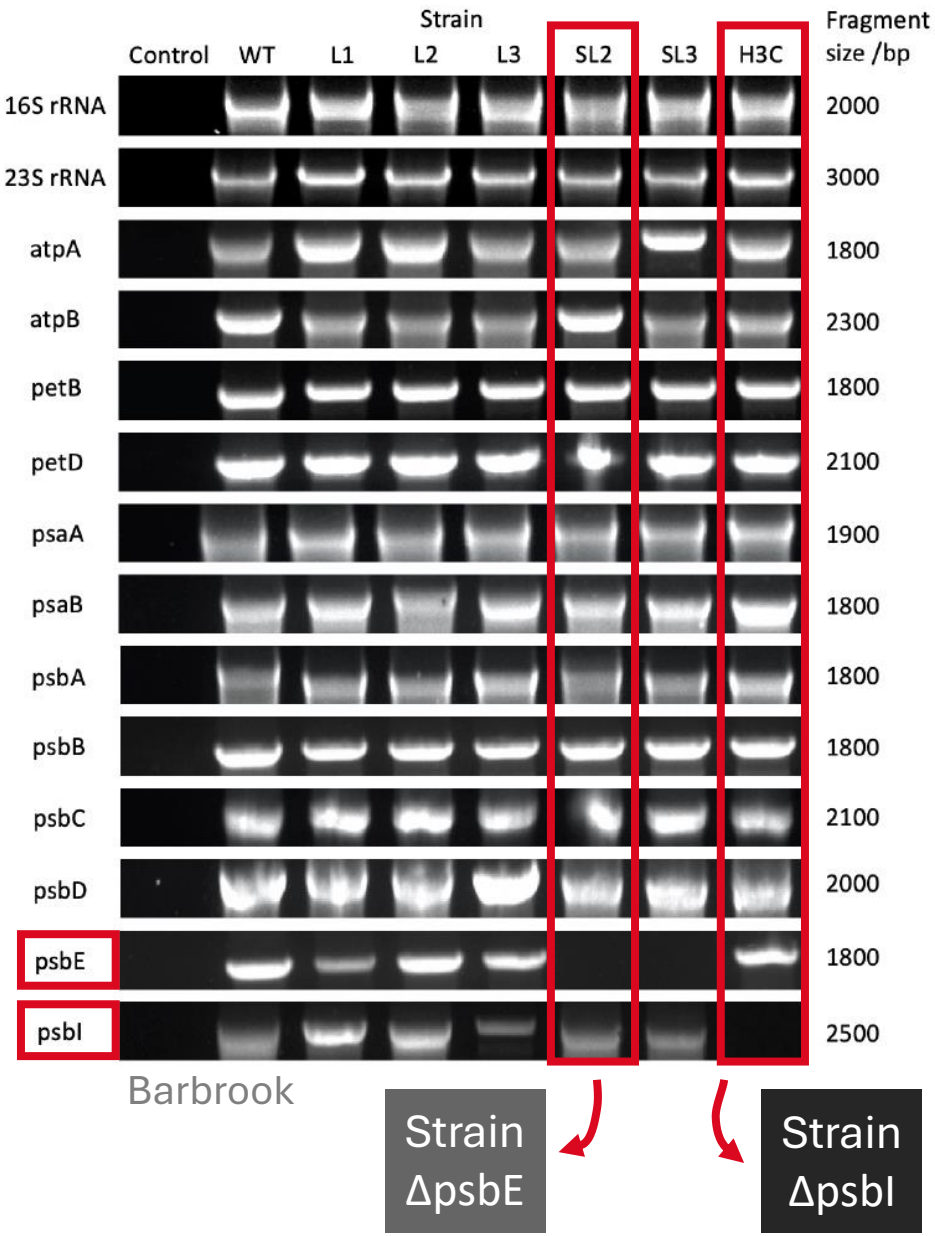




Barbrook

Adrian Barbrook, Ellen Nisbet, Christopher Howe

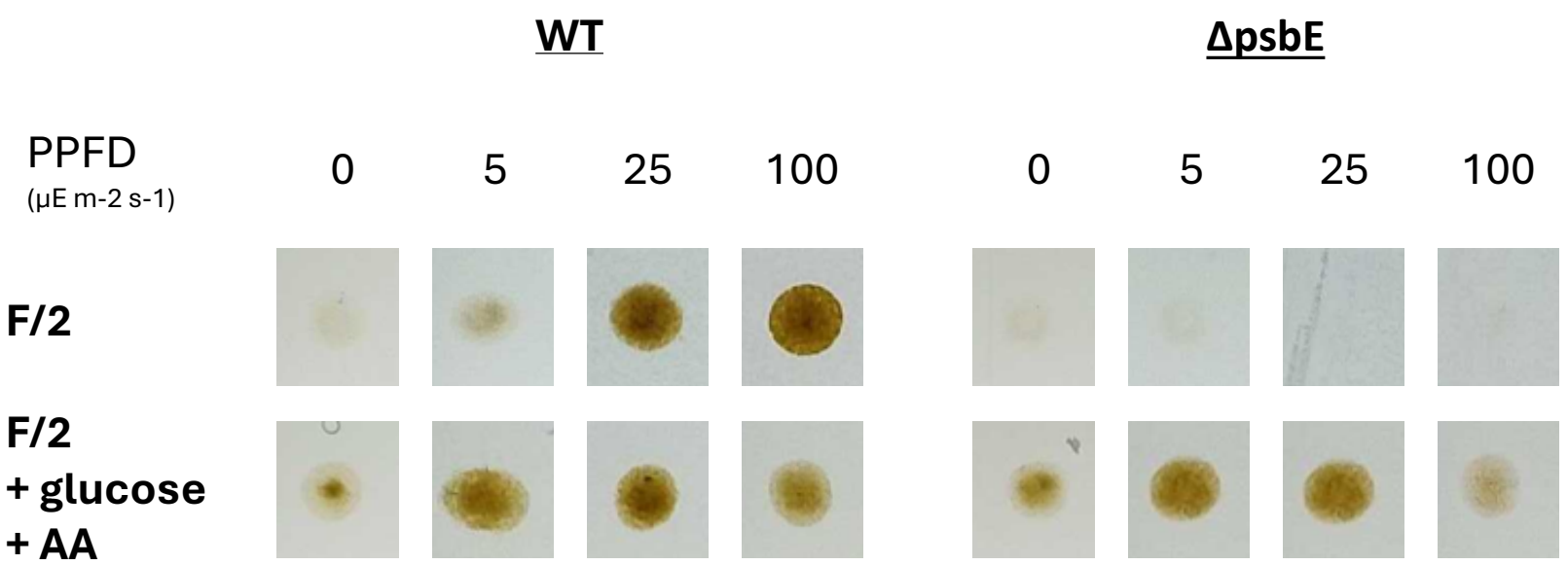




WT

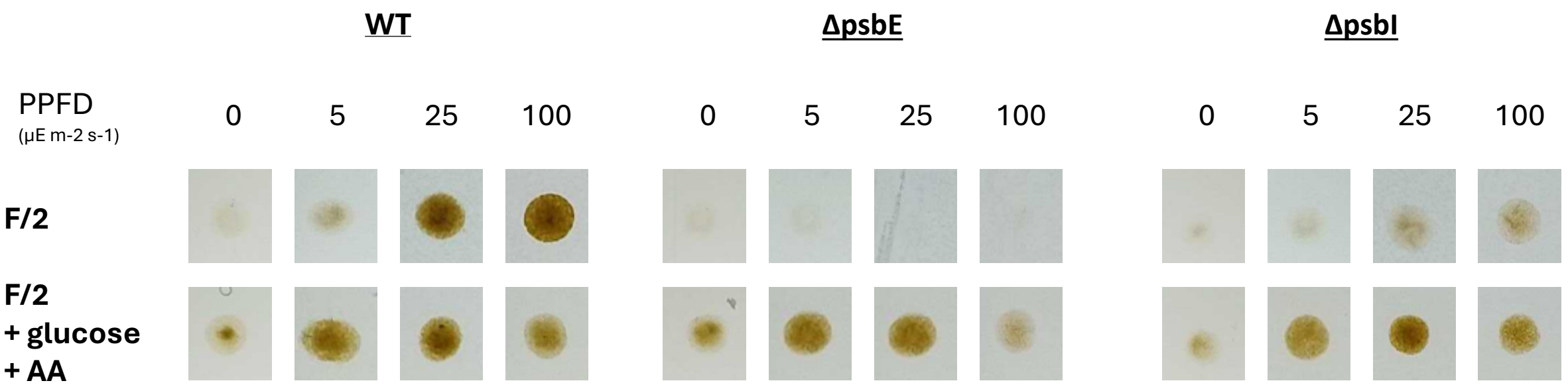
PPFD (µE m-2 s-1)	0	5	25	100
F/2				
F/2 + glucose + AA				

3. **Results** – Growth on plates



→ No growth in F/2
→ Growth with gluc & AA
but photosensitivity

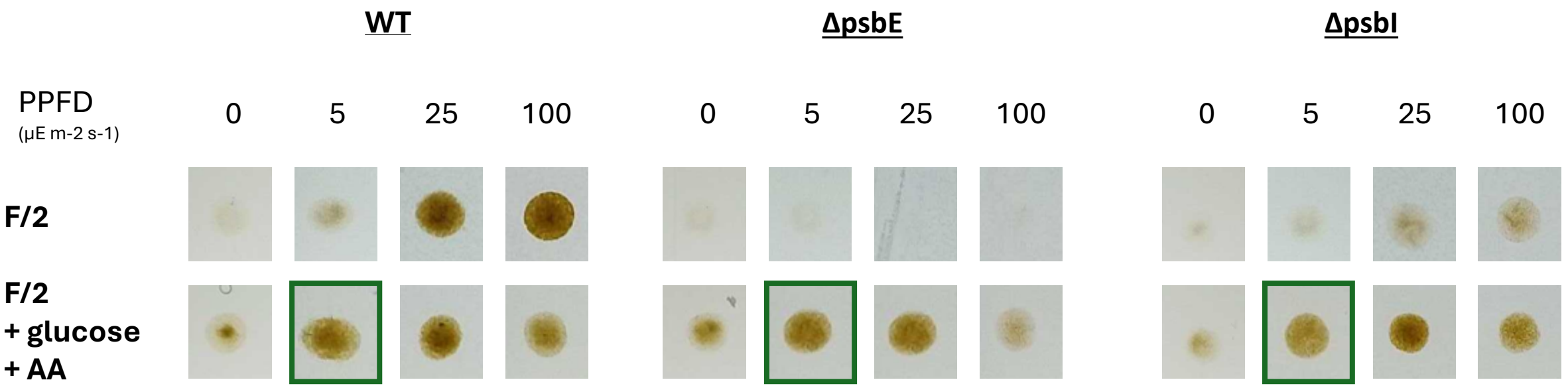
3. **Results** – Growth on plates



→ No growth in F/2
→ Growth with gluc & AA but photosensitivity

→ Impaired growth in F/2
→ Growth with gluc & AA but photosensitivity

3. **Results** – Growth on plates

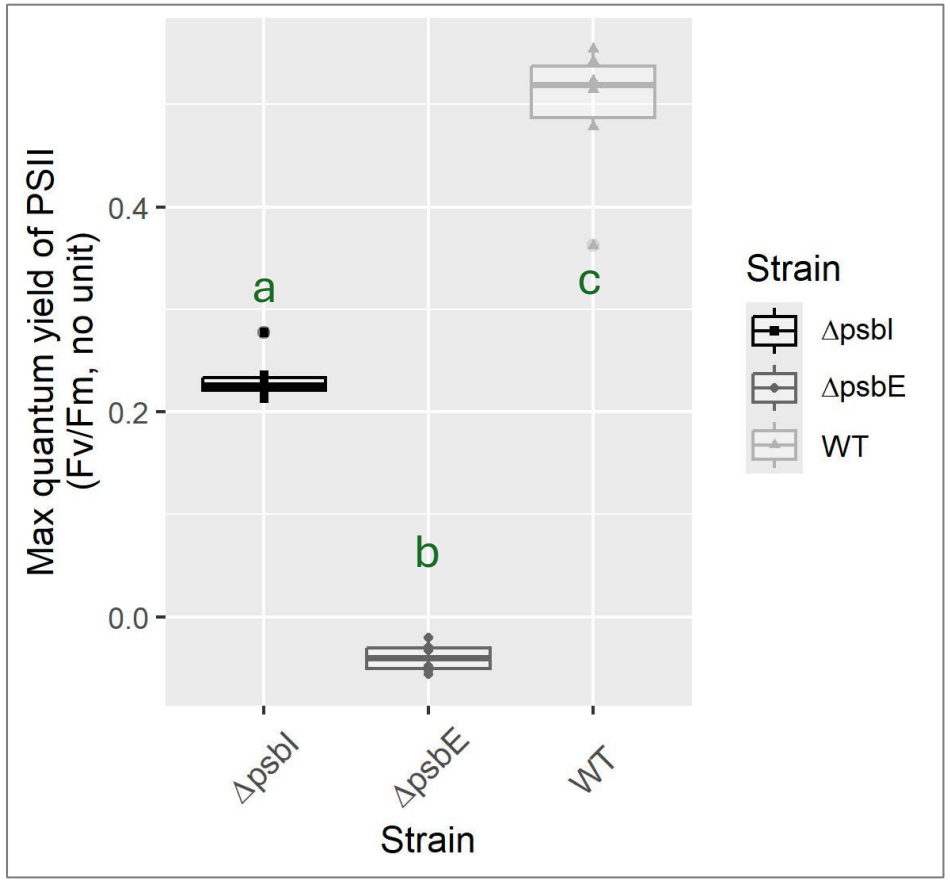


→ No growth in F/2
→ Growth with gluc & AA but photosensitivity

→ Impaired growth in F/2
→ Growth with gluc & AA but photosensitivity

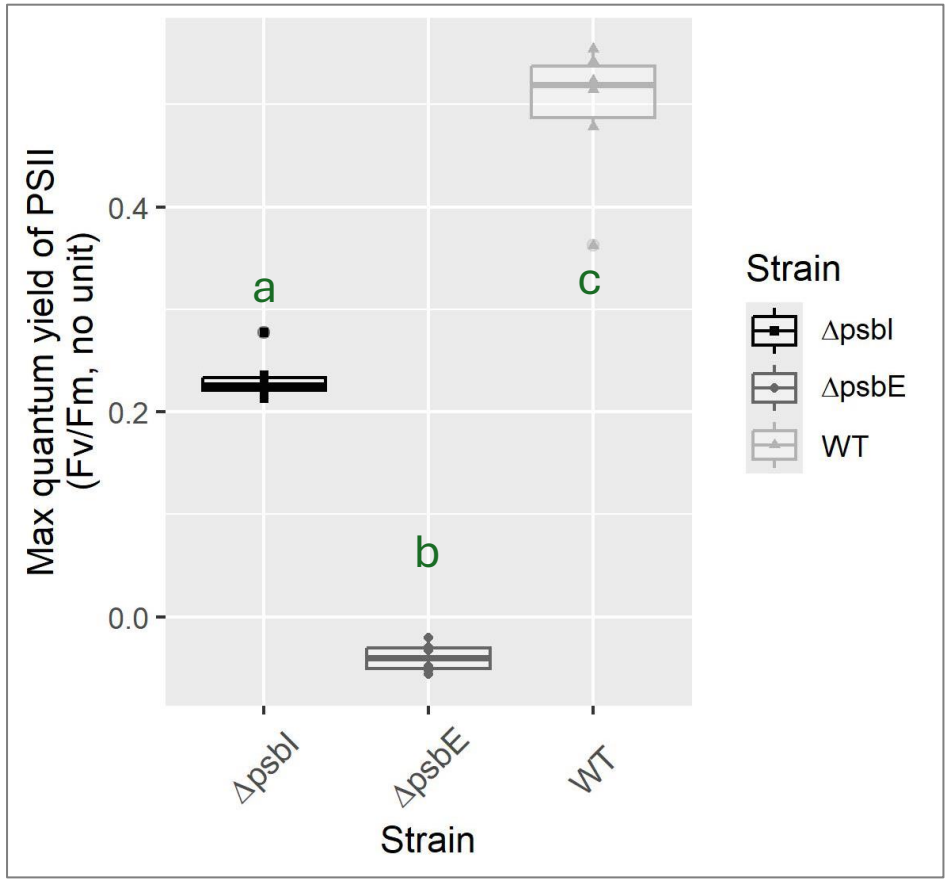
→ Functional PSI and PSII analyses

Maximum quantum yield of PSII



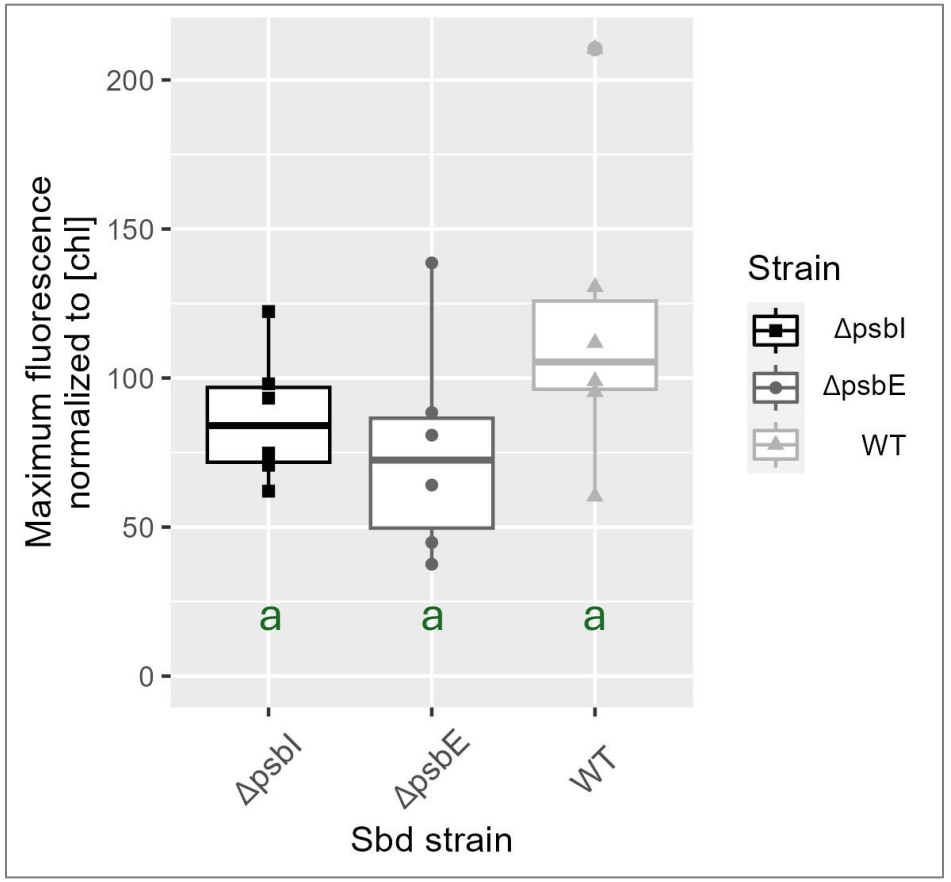
→ Non functional PSII in ΔpsbE strain
→ Impaired PSII in ΔpsbI strain

Maximum quantum yield of PSII



→ Non functional PSII in ΔpsbE strain
→ Impaired PSII in ΔpsbI strain

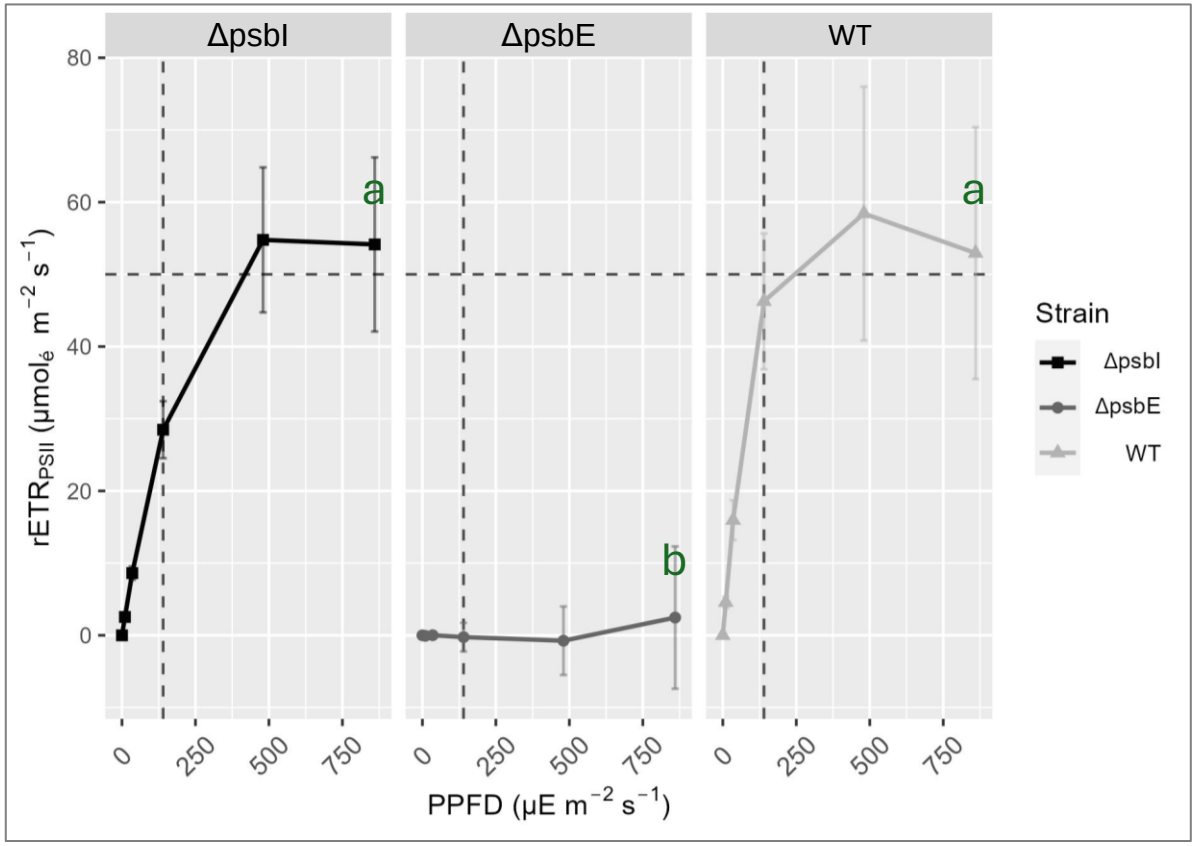
Maximum fluorescence of PSII



→ Partially assembled but non-functional PSII in ΔpsbE strain ?

3. **Results** – PSII – Relative electron transport rate

Relative electron transport rate through PSII



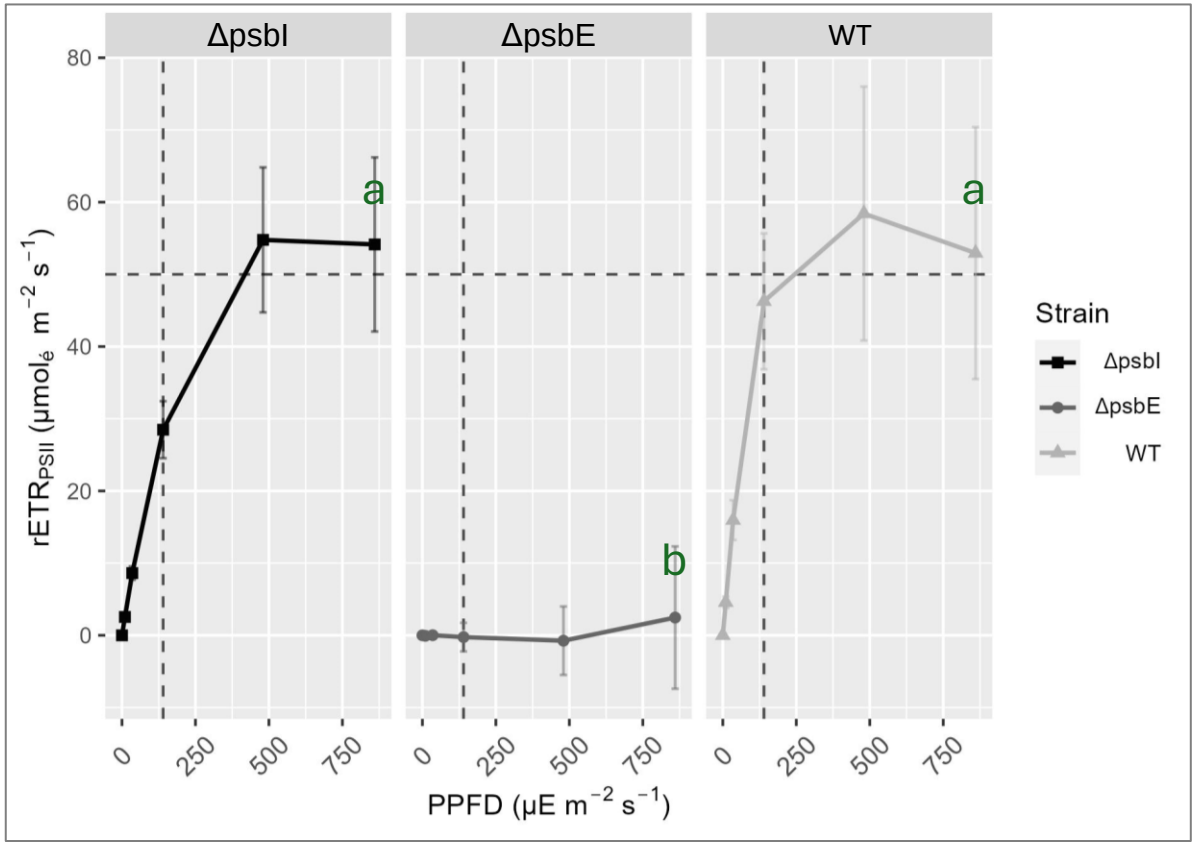
→ No PSII activity in ΔpsbE

→ Sustained maximum activity of PSII for ΔpsbI

3. **Results** – PSII – Relative electron transport rate

3 s light steps

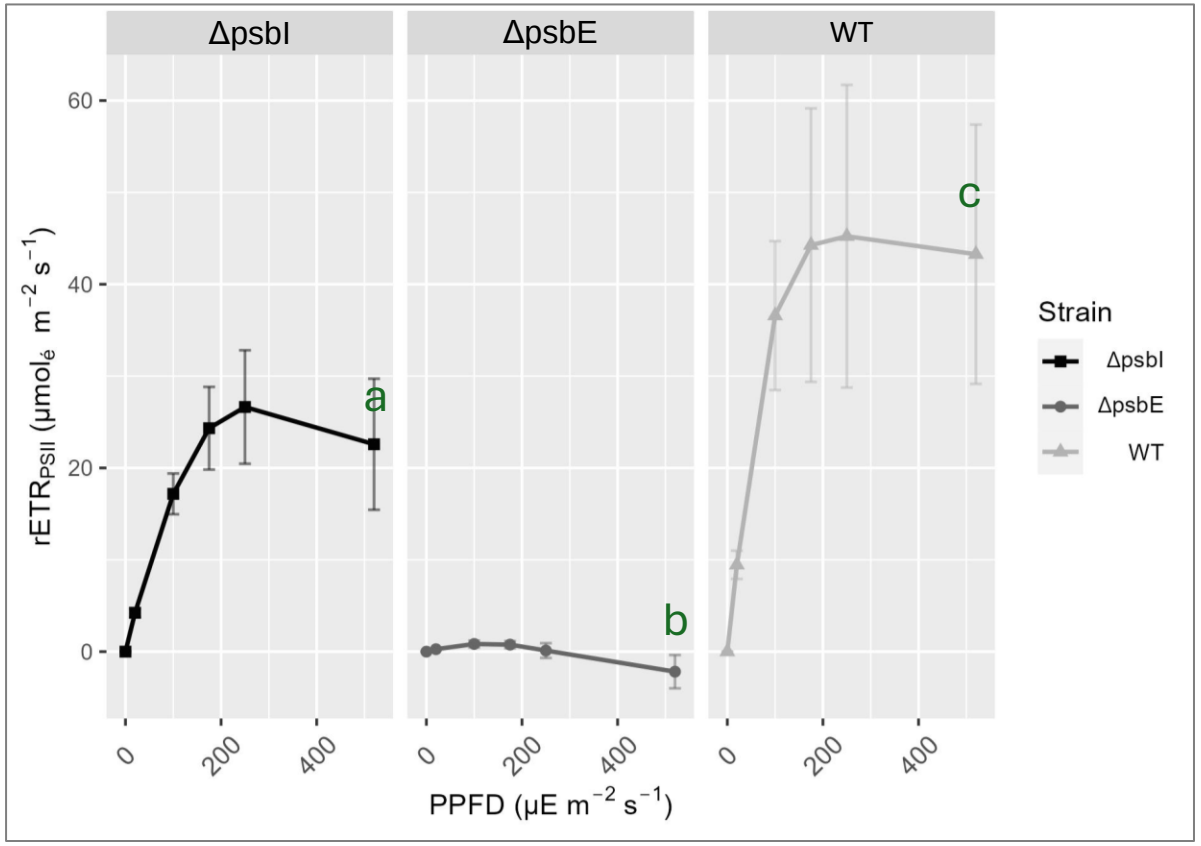
Relative electron transport rate through PSII



→ No PSII activity in ΔpsbE
→ Sustained maximum activity of PSII for ΔpsbI

3 min light steps

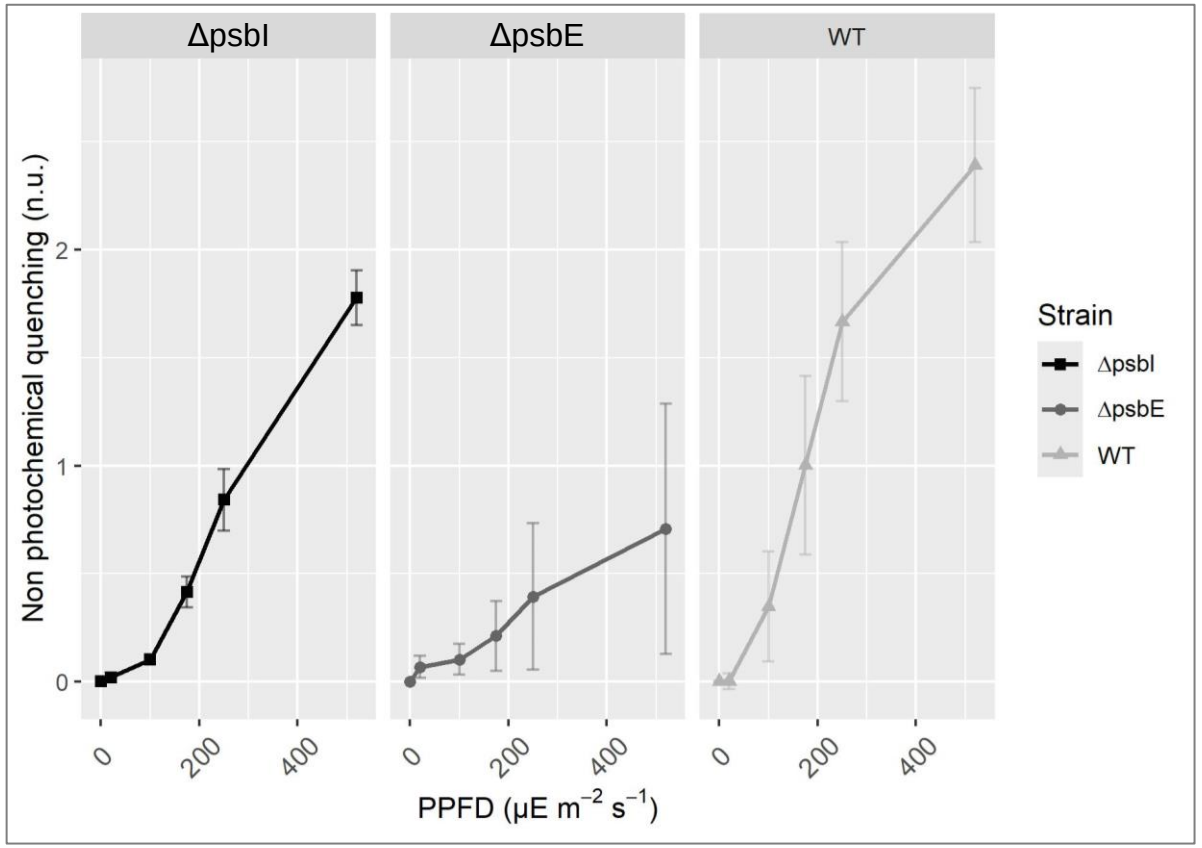
Relative electron transport rate through PSII



→ No PSII activity in ΔpsbE
→ Lower activity of PSII for ΔpsbI

3 min light steps

Non photochemical quenching ~ PPFD

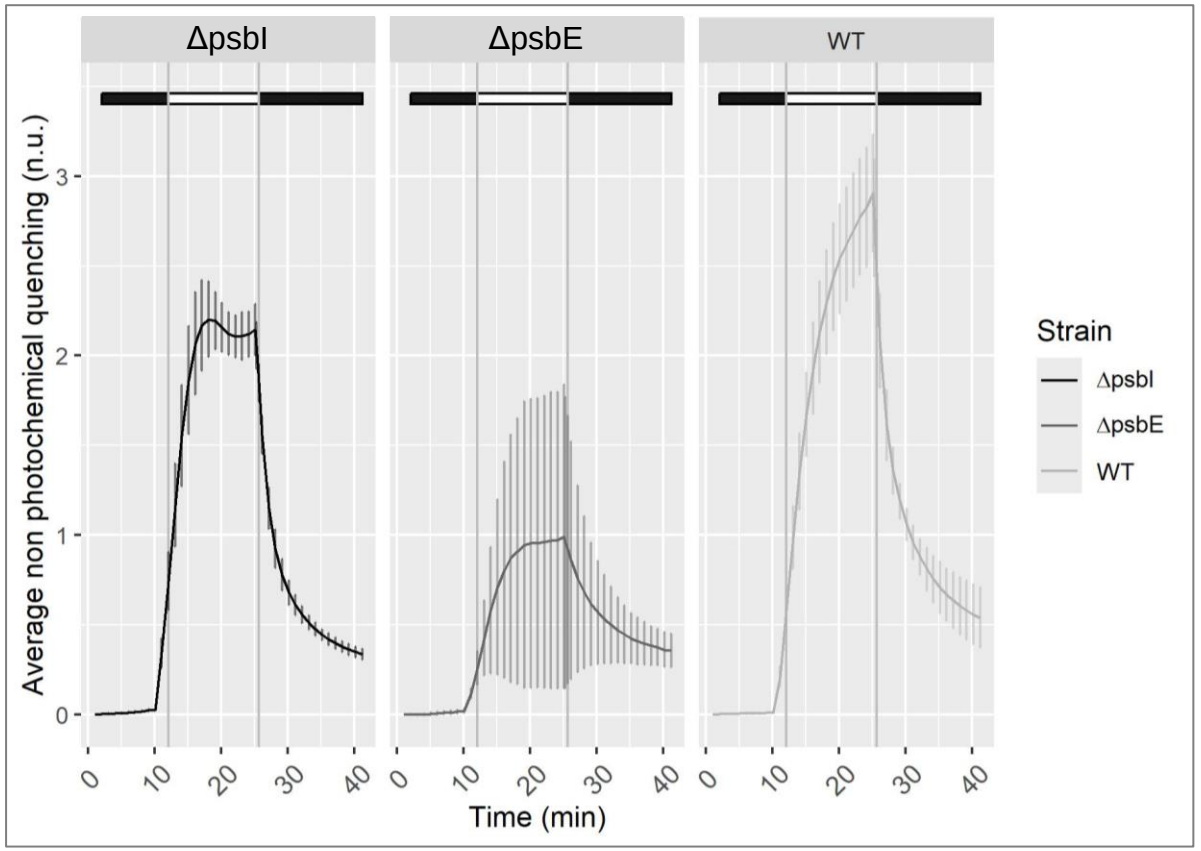


→ Light-dependent NPQ in ΔpsbE

3. **Results** – PSII – Non photochemical quenching

10 min light step

Non photochemical quenching ~ time

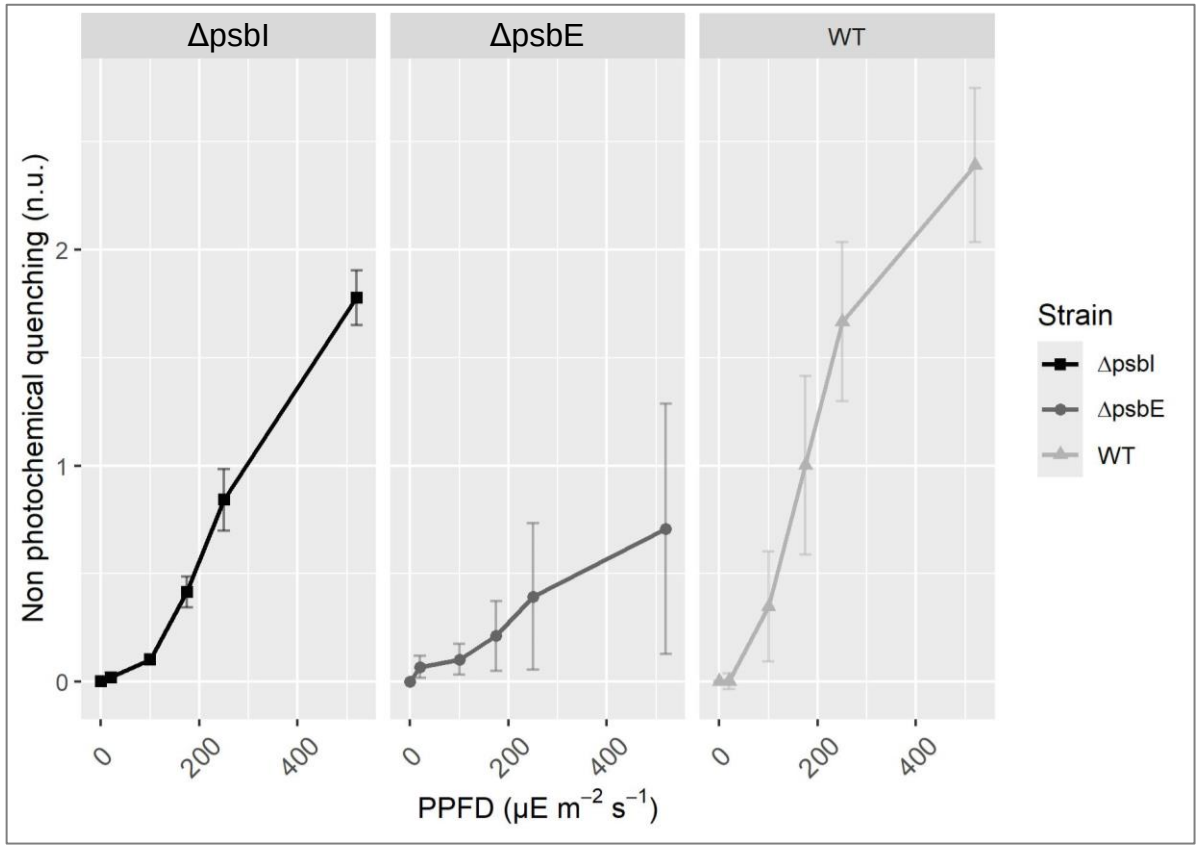


→ Limited NPQ capacity in ΔpsbI strain in long term

→ Reversible NPQ in all strains → ΔpH dependent

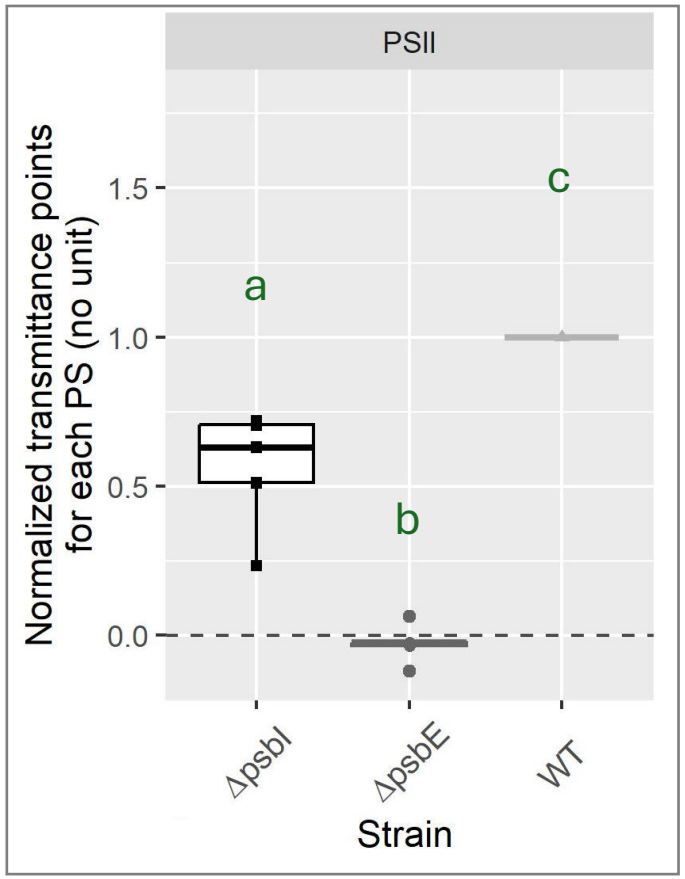
3 min light steps

Non photochemical quenching ~ PPFD

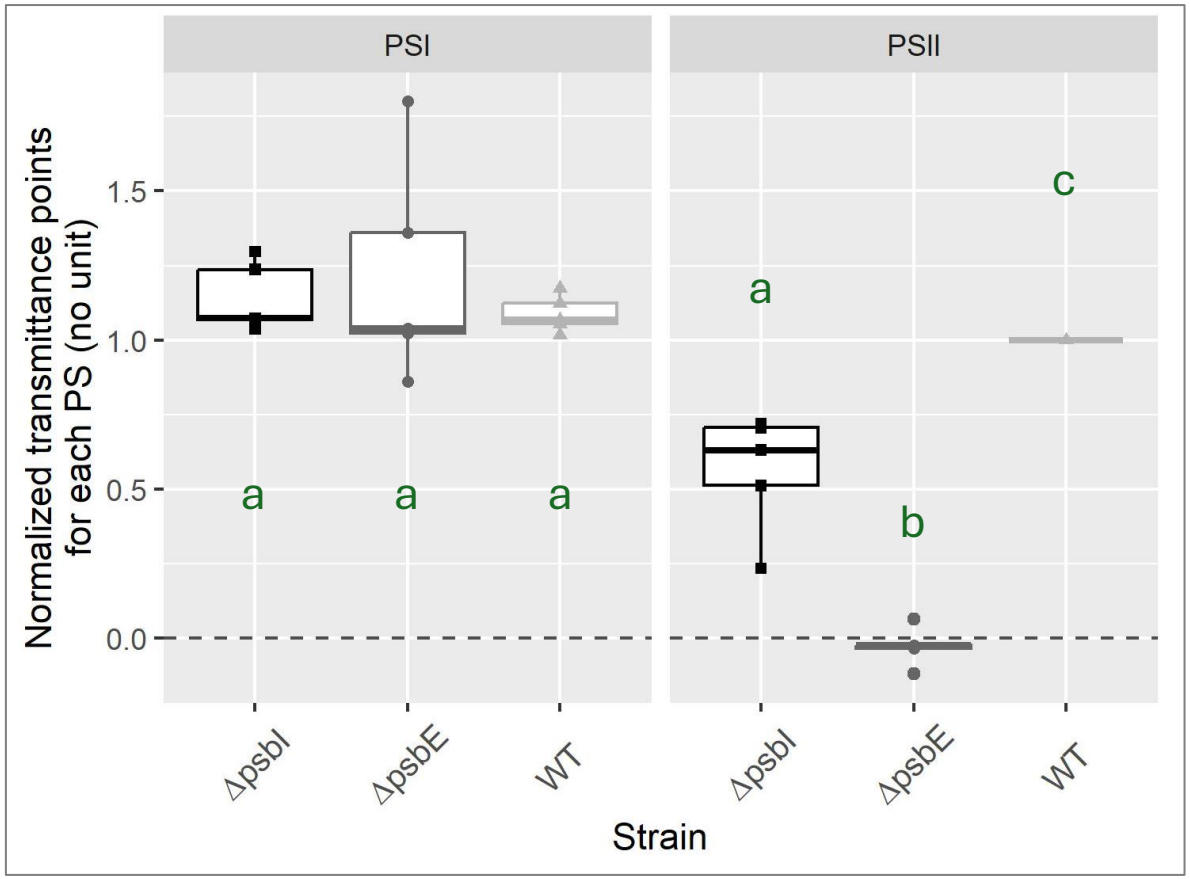


→ Light-dependent NPQ in ΔpsbE

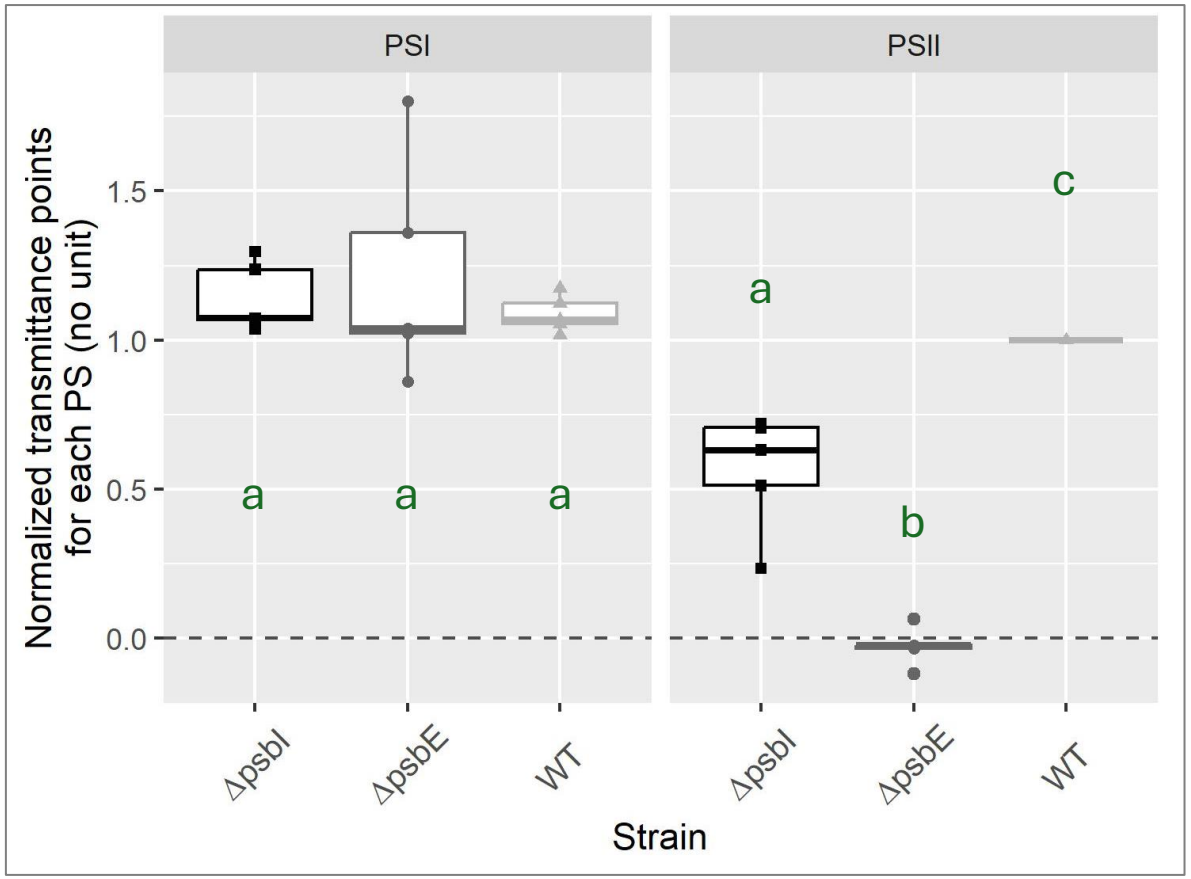
Relative amount of active PS (ECS)



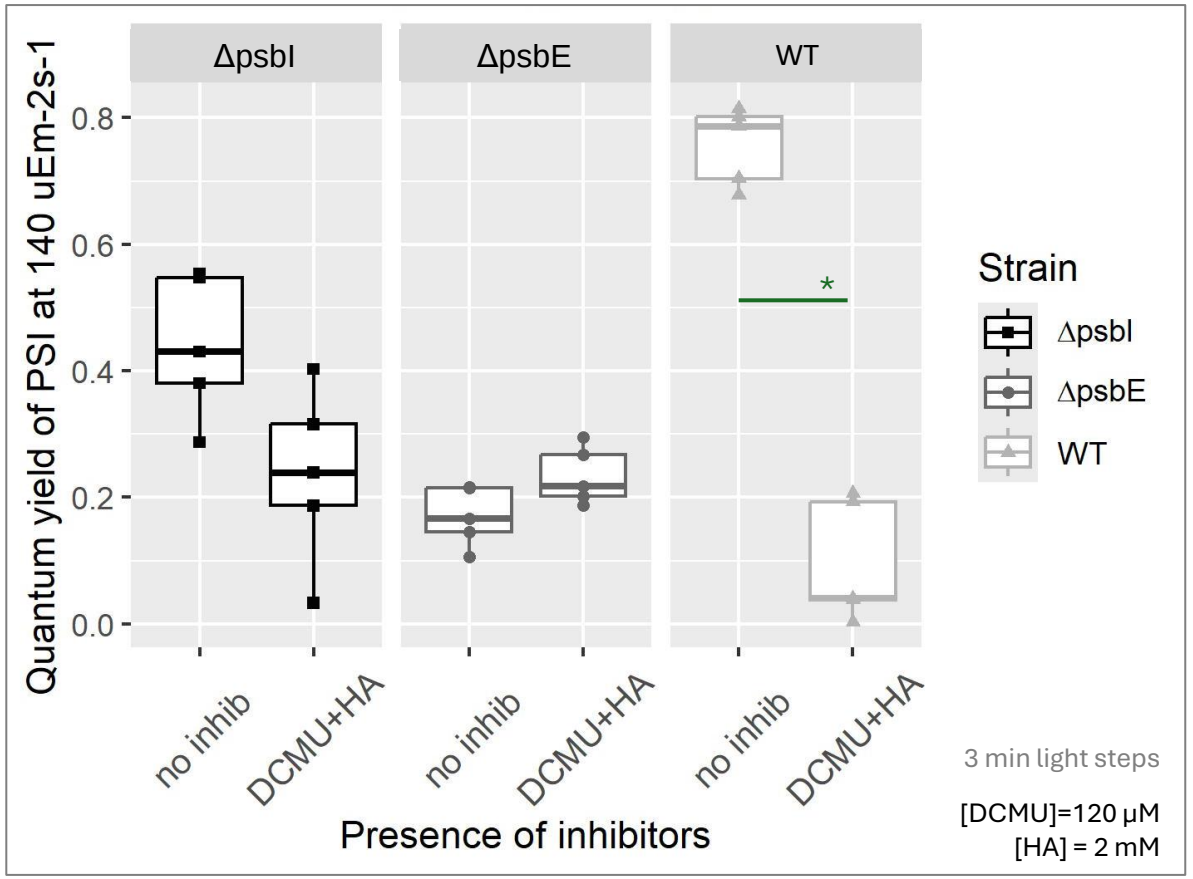
Relative amount of active PS (ECS)



Relative amount of active PS (ECS)



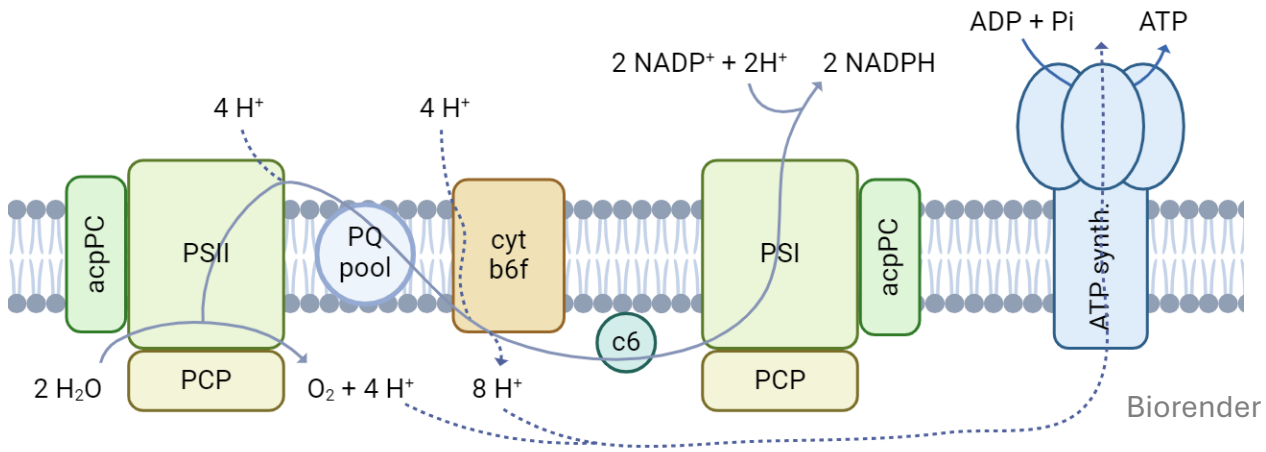
Quantum yield of PSI



→ PSII-independent PSI activity in ΔpsbE mutants

→ CEF-PSI

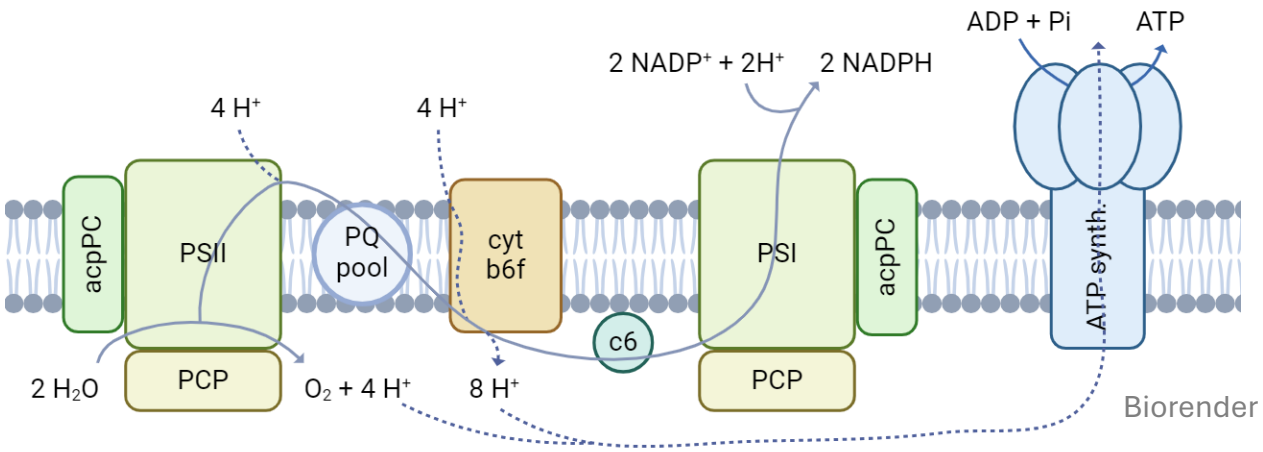
4. Conclusion



ΔpsbI — Fv/Fm and PSII activity ↘ + photosensitivity; PSI activity ≈ → Similar phenotype in *C. reinhardtii*, tobacco and Synechocystis (Dobakova 2007)

ΔpsbE — Non functional fluo emitting PSII structures; PSII-independent PSI activity → Similar phenotype in *C. reinhardtii* (Morais 1998)

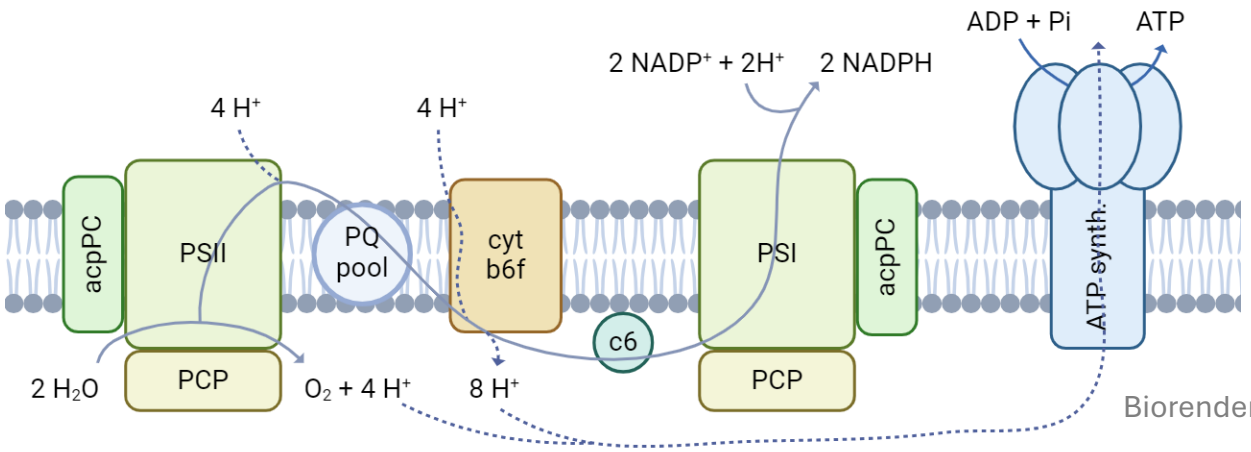
4. Conclusion



Photosynthesis in *Symbiodinium*

ΔpsbI	Fv/Fm and PSII activity ↘ + photosensitivity	PSI activity ≈	→	Similar phenotype in *C. reinhardtii*, tobacco and Synechocystis (Dobakova 2007)
ΔpsbE	Non functional fluo emitting PSII structures	PSII-independent PSI activity	→	Similar phenotype in *C. reinhardtii* (Morais 1998)

4. Conclusion



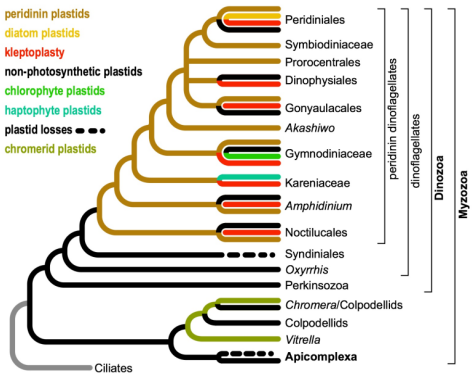
Biorender

Photosynthesis in *Symbiodinium*

ΔpsbI — Fv/Fm and PSII activity ↘ + photosensitivity; PSI activity ≈ → Similar phenotype in *C. reinhardtii*, tobacco and Synechocystis (Dobakova 2007)

ΔpsbE — Non functional fluo emitting PSII structures; PSII-independent PSI activity → CEF-PSI → ATP production → Similar phenotype in *C. reinhardtii* (Morais 1998)

Loss of photosynthesis in dinoflagellates



Waller 2017 (6)

Thanks to my colleagues

- Pierre Cardol

- Emma Comté
- Sarah Joly

- Yanis Aoudache
- Tom Feller
- Hadrien Forêt
- Alain Gervasi
- Antoine Kairis
- Pablo Perez

Thanks to our collaborators

- Ellen Nisbet (School of Biosciences, University of Nottingham, UK)
- Christopher Howe (Department of Biochemistry, University of Cambridge, UK)
- Adrian Barbrook (Department of Biochemistry, University of Cambridge, UK)

Thanks for your attention!

Edmee.Royen@doct.uliege.be

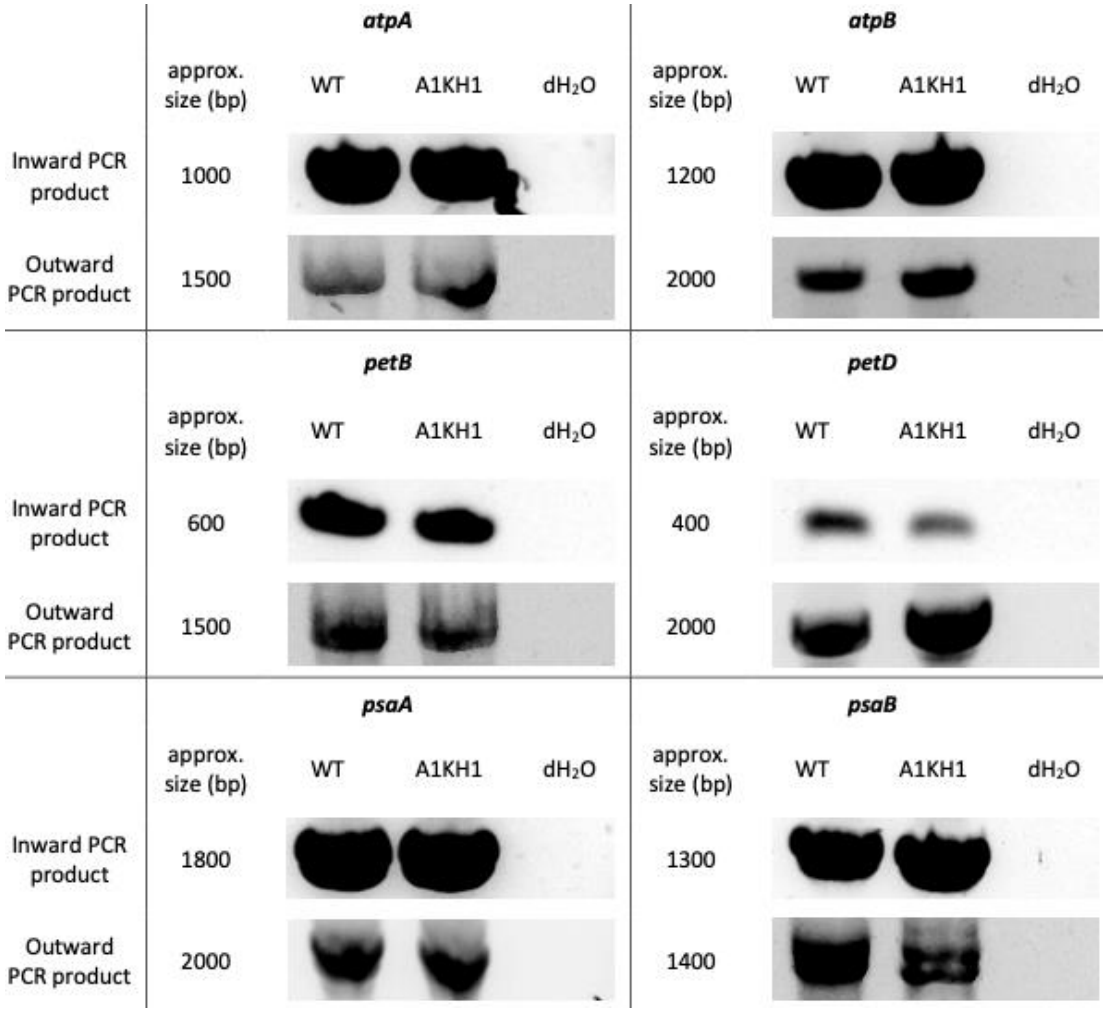
References

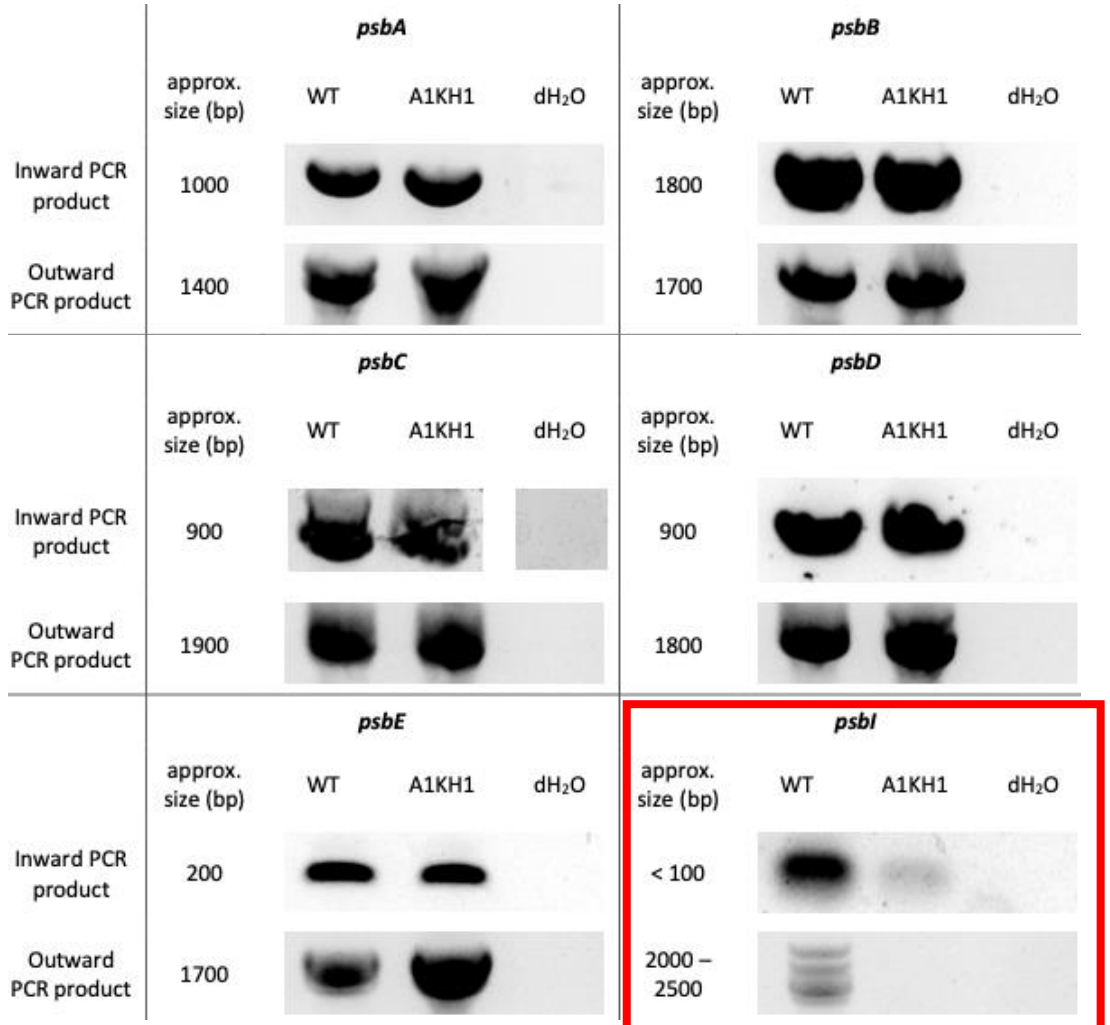
1. Baums, Iliana & Devlin-Durante, Meghann & LaJeunesse, Todd. (2015). Baums et al. 2014 MOLECECOL.
2. Oxford Scientific, “Coral pocillopora sp. expanded polyps with symbiotic zooxanthellae”, Getty Image, accessed on 20/02/22, illegally available at https://ocean.si.edu/ocean-life/invertebrates/zooxanthellae-and-coral-bleaching
3. Kieran Cox, “An Acropora colony on Christmas Island, Kiribati, before bleaching that struck the reef in 2015 and 2016”, National Geographic, accessed on 12/03/24, available at https://www.nationalgeographic.com/science/article/coral-bleaching-reefs-climate-change-el-nino-environment
4. Hae Jin Jeong et al (2012). Heterotrophic feeding as a newly identified survival strategy of the dinoflagellate *Symbiodinium*. Proceedings of the National Academy of Sciences. (vol. 109 | no. 31)
5. Tintinnidguy, “Microzooplankton, the major grazers of the plankton: spiny-globe Protoperidinium dinoflagellate. From the Thau Lagoon of Sète, France”, Wikipedia, accessed on 12/03/24, available at https://en.wikipedia.org/wiki/Protoperidiniaceae#/media/File:Protoperidinium_dinoflagellate.jpg
6. Ross Waller and Luděk Kořený (2017). Plastid complexity in dinoflagellates: a picture of gains, losses, replacements and revisions. Cambridge University Library.
7. FWC Fish and Wildlife Research Institute, “*Alexandrium monilatum* (light micrograph)”, Flickr, accessed on 12/03/24, available at https://flickr.com/photos/myfwc/8621534507
8. Shah Mahfuzur and Joon-Baek Lee (2013). Presence of benthic dinoflagellates around coastal waters of Jeju Island including newly recorded species. Journal of Ecology and Environment. 36 (4).
9. Wim van Egmond, “Ceratium hirundinella (freshwater dinoflagellate), living specimen”, Nikon’s Small World, accessed on 12/03/24, available at https://www.nikonsmallworld.com/galleries/2009-photomicrography-competition/ceratium-hirundinella-freshwater-dinoflagellate-living-specimen
10. Tintinnidguy, “Ornithocercus magnificus”, Flickr, accessed on 12/03/24, available at https://www.flickr.com/photos/56879865@N08/13954604481/in/photostream/
11. Dariusz Niedzwiedzki et al. (2014). Spectroscopic properties of the Chlorophyll a–Chlorophyll c2–Peridinin-Protein-Complex (acpPC) from the coral symbiotic dinoflagellate *Symbiodinium*. Photosynthesis Research. 120:125–139
12. Adrian C. Barbrook, Christopher J. Howe & R. Ellen R. Nisbet (2018). Breaking up is hard to do: the complexity of the dinoflagellate chloroplast genome. Perspectives in Phycology 6 (1-2)

References

13. Marika Dobakova et al. (2007). Role of the PsbI Protein in Photosystem II Assembly and Repair in the Cyanobacterium Synechocystis sp. PCC 6803. Plant Physiology. Vol. 145, pp. 1681–1691
14. Morais et al. (1998). The Chloroplast-encoded a Subunit of Cytochrome b-559 Is Required for Assembly of the Photosystem Two Complex in both the Light and the Dark in Chlamydomonas reinhardtii. The Journal of Biological Chemistry. Vol. 273, No. 45

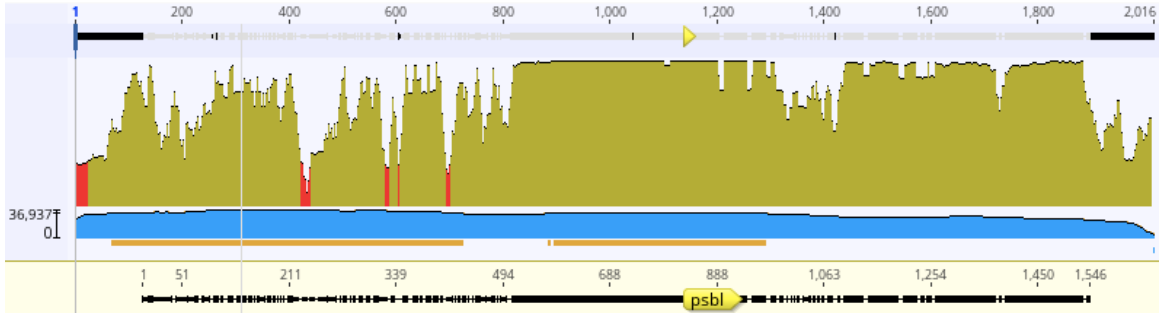
PCR amplification of coding minicircles with WT and A1KH1 DNA



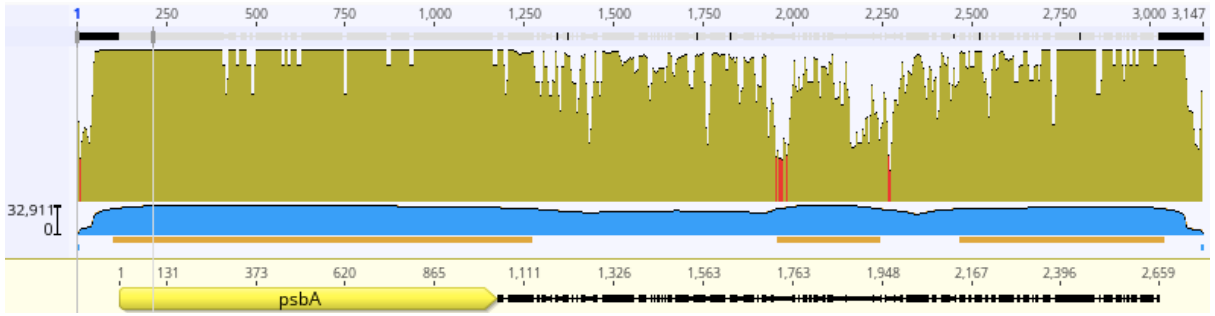


DNA sequencing shows absence of *psbI* coding regions in A1KH1 extracts

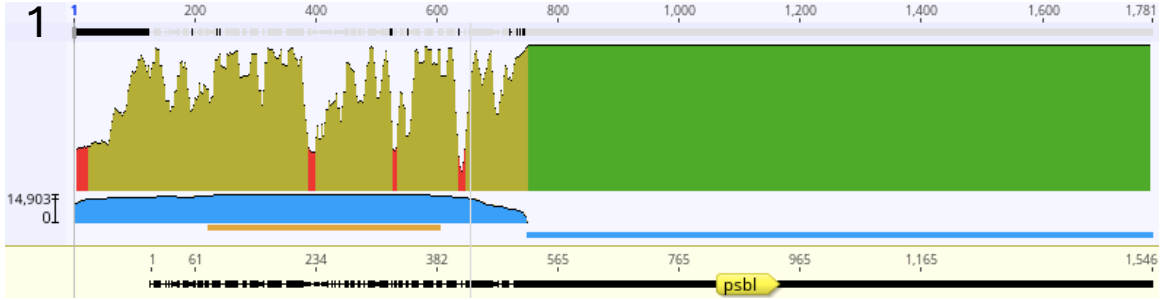
wt



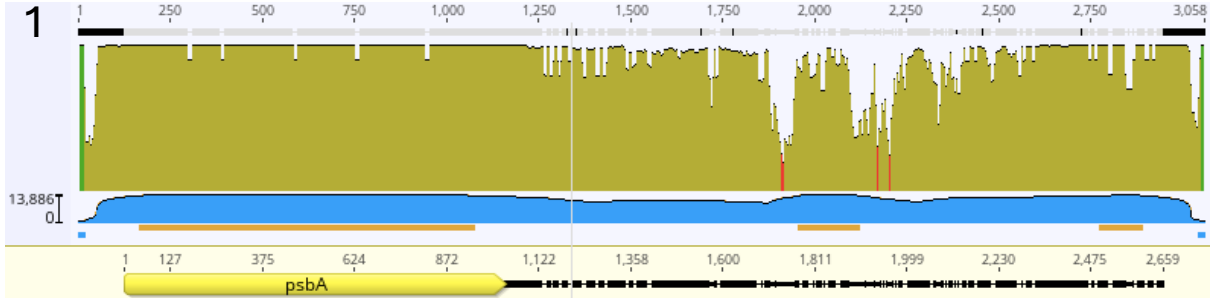
wt



A1KH



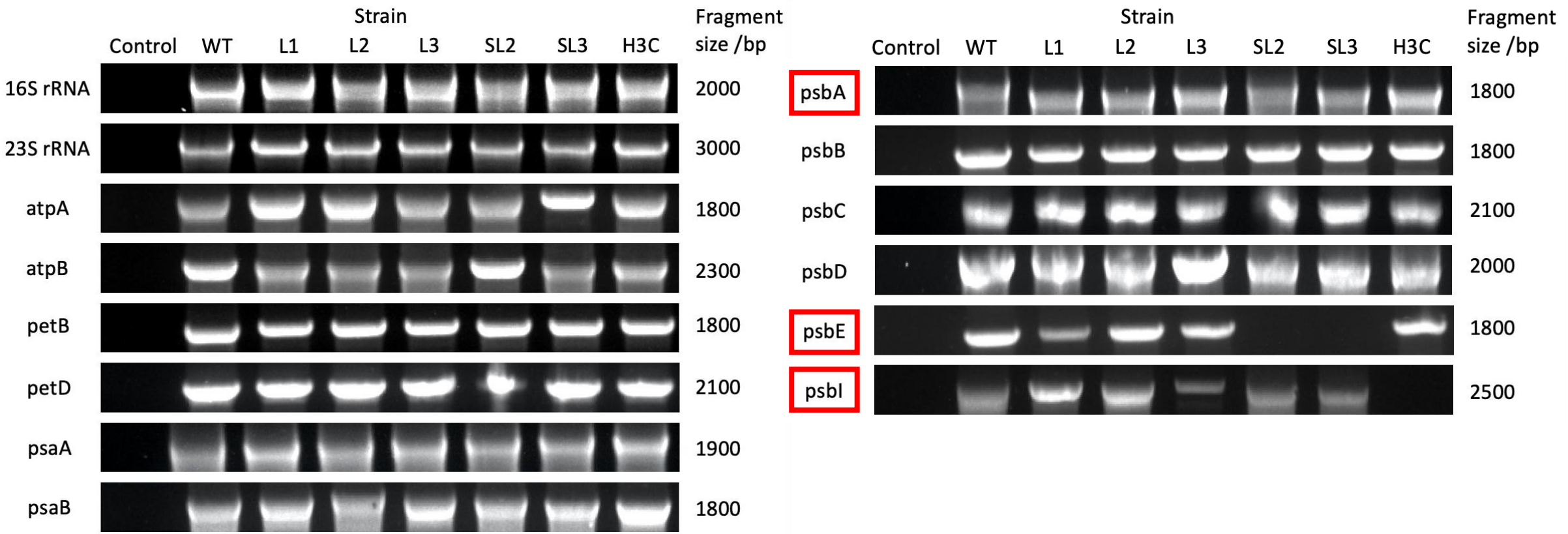
A1KH



DNA reads matched to *psbI* minicircle

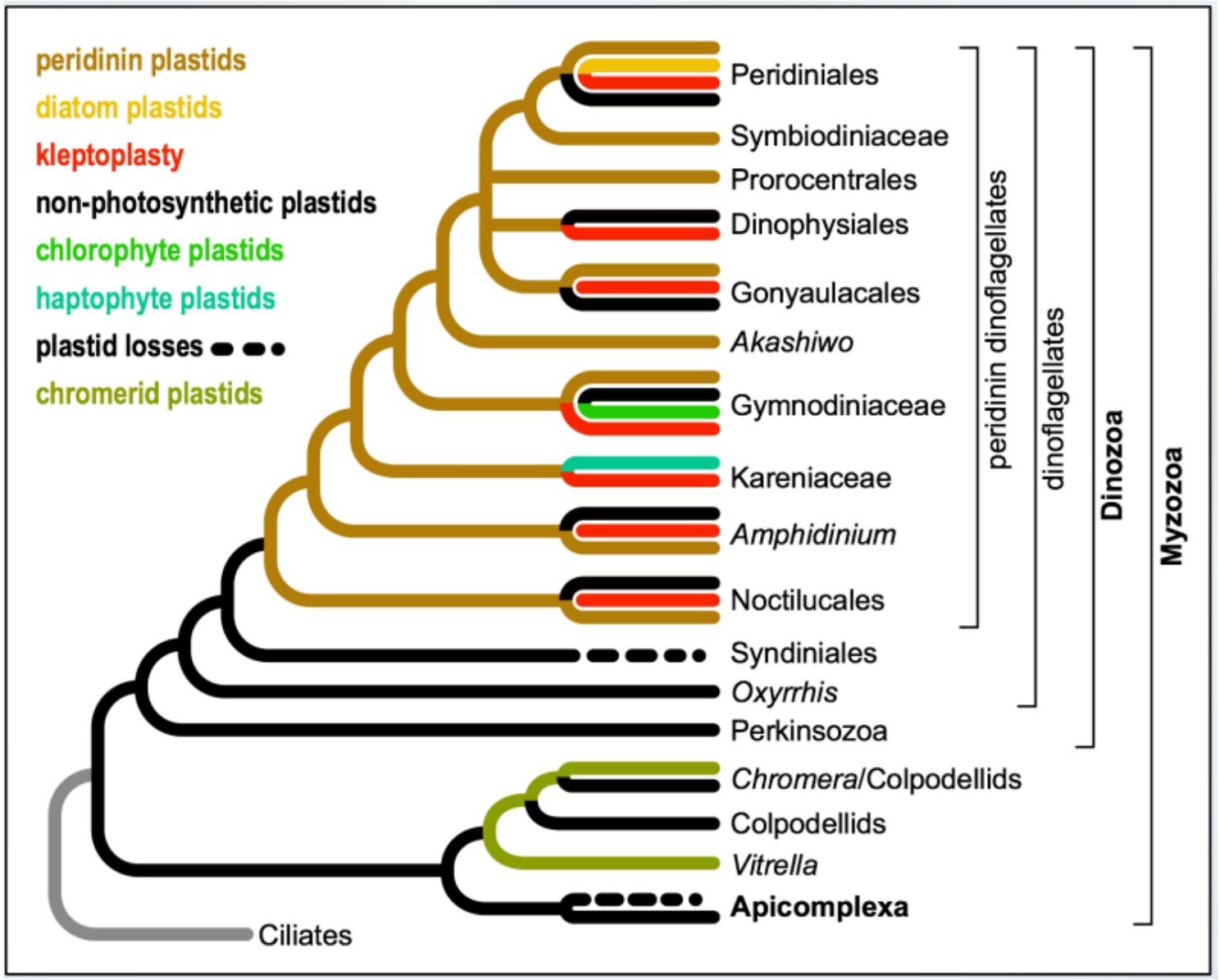
DNA reads matched to *psbA* minicircle

Other strains – outfacing PCRs

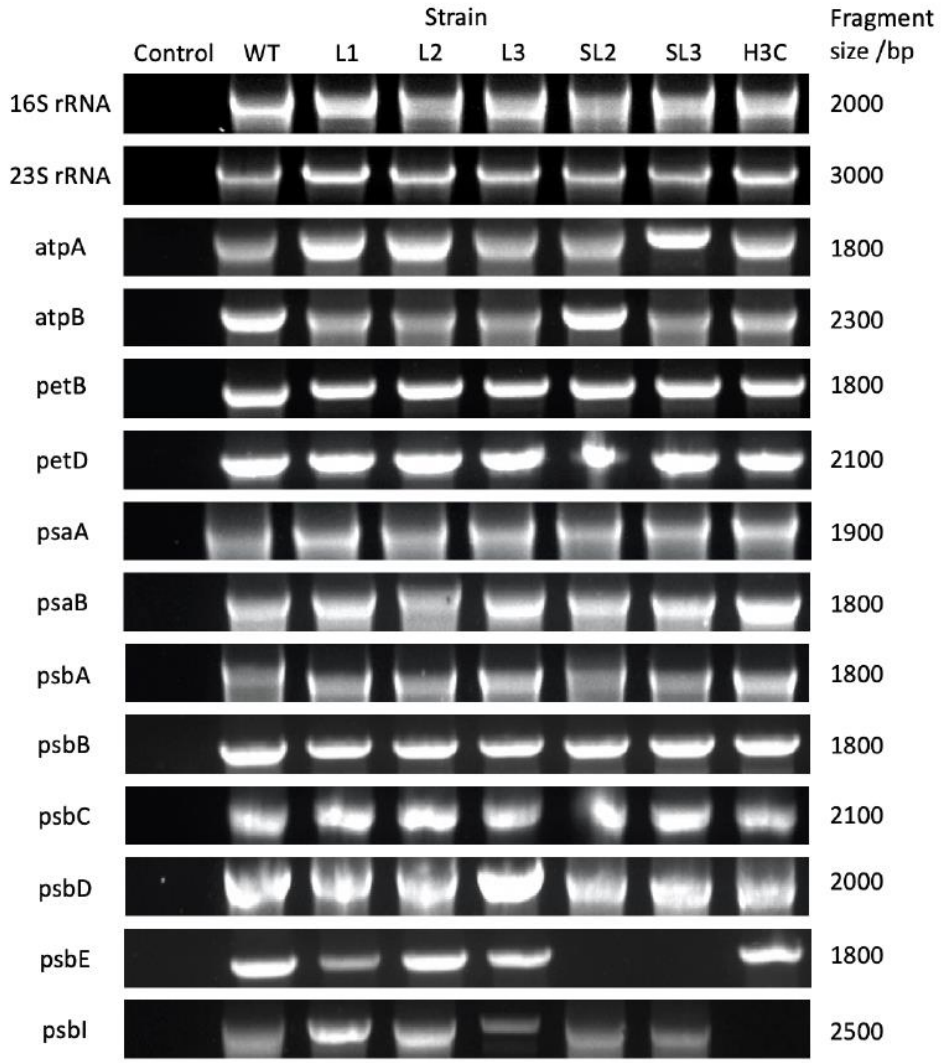


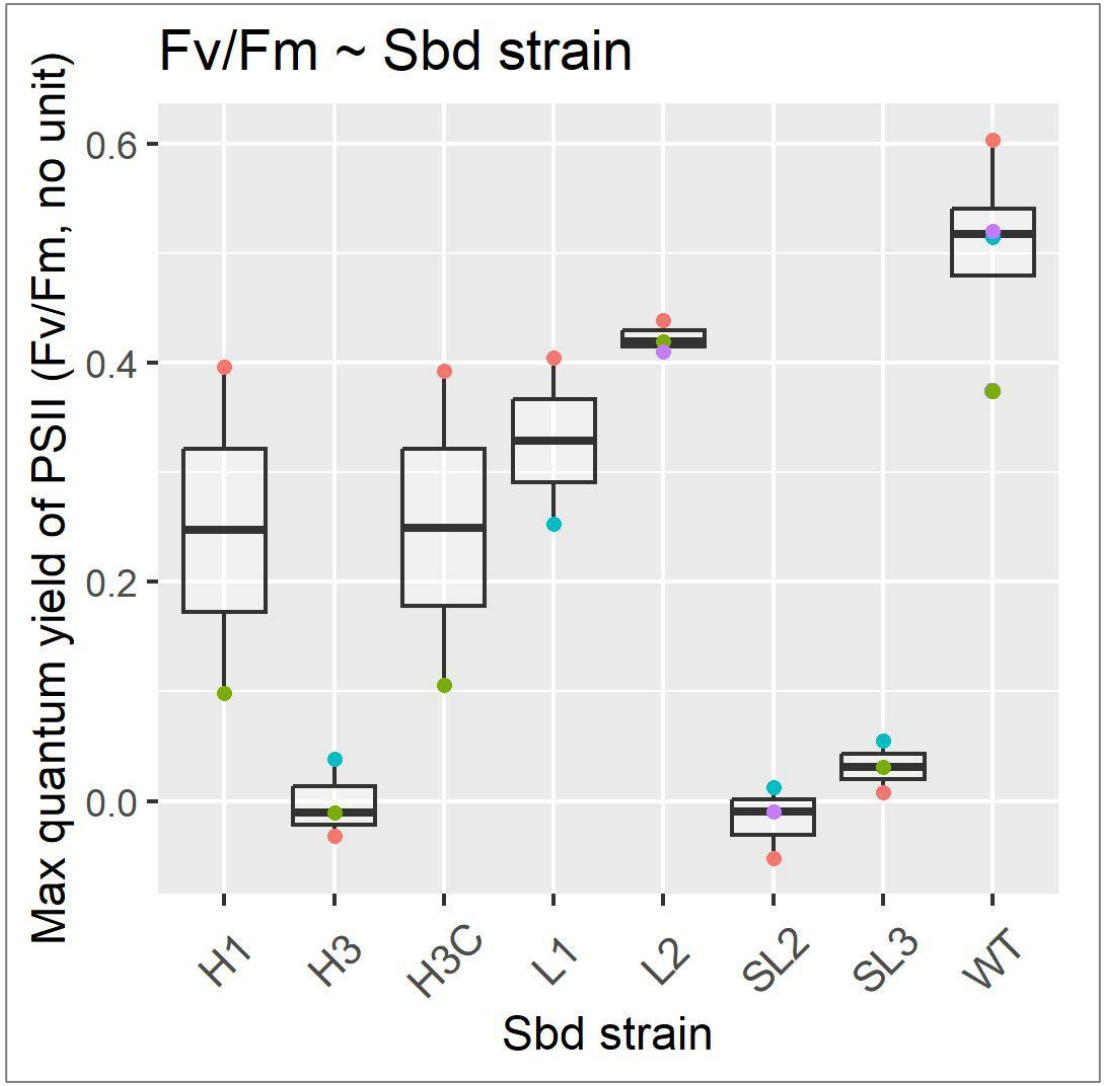
1. Introduction

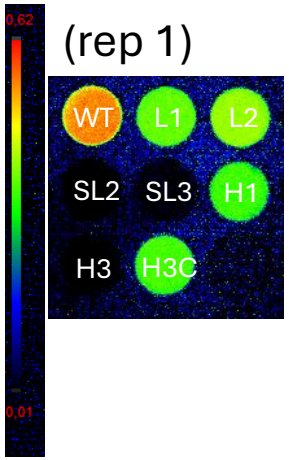
Full dinos & friends tree (Waller 2017)

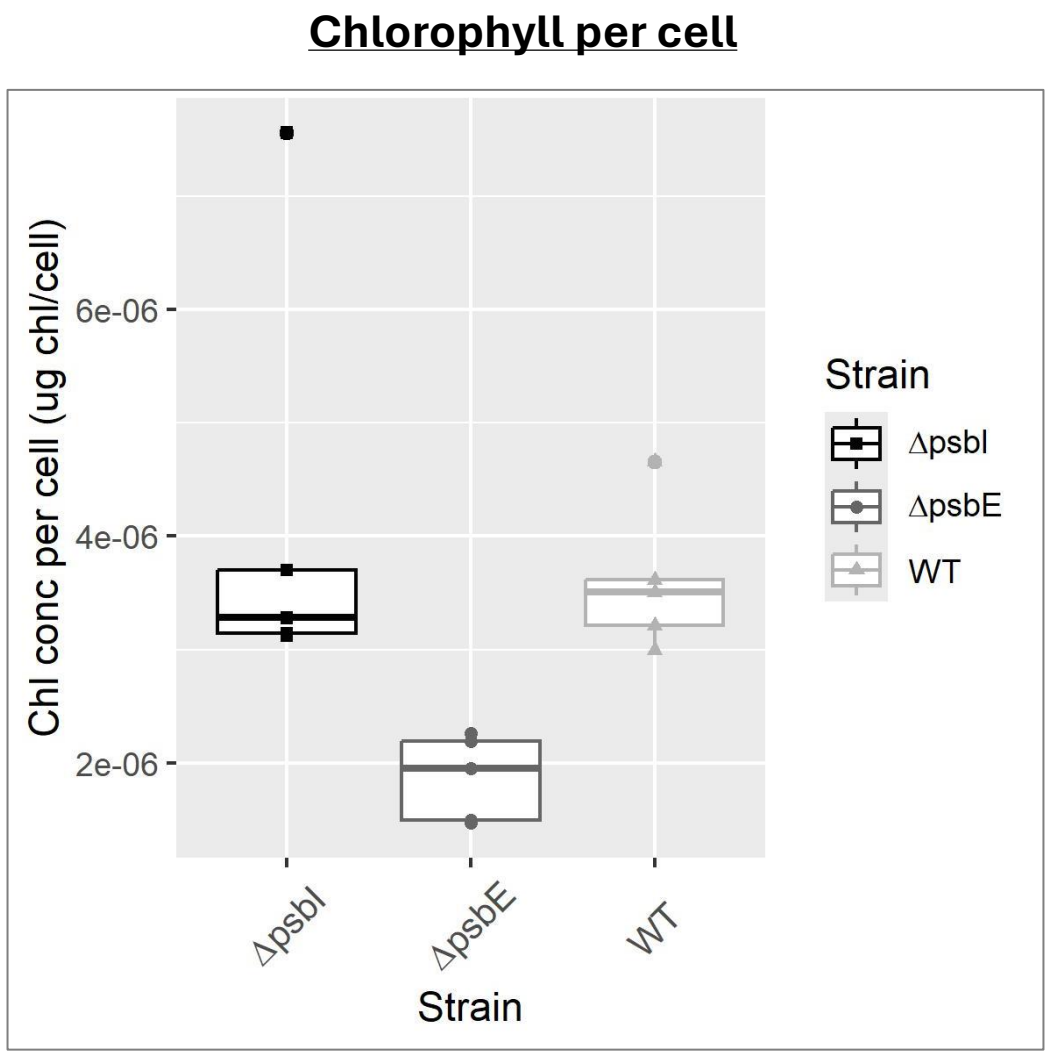


3. **Results** – General features

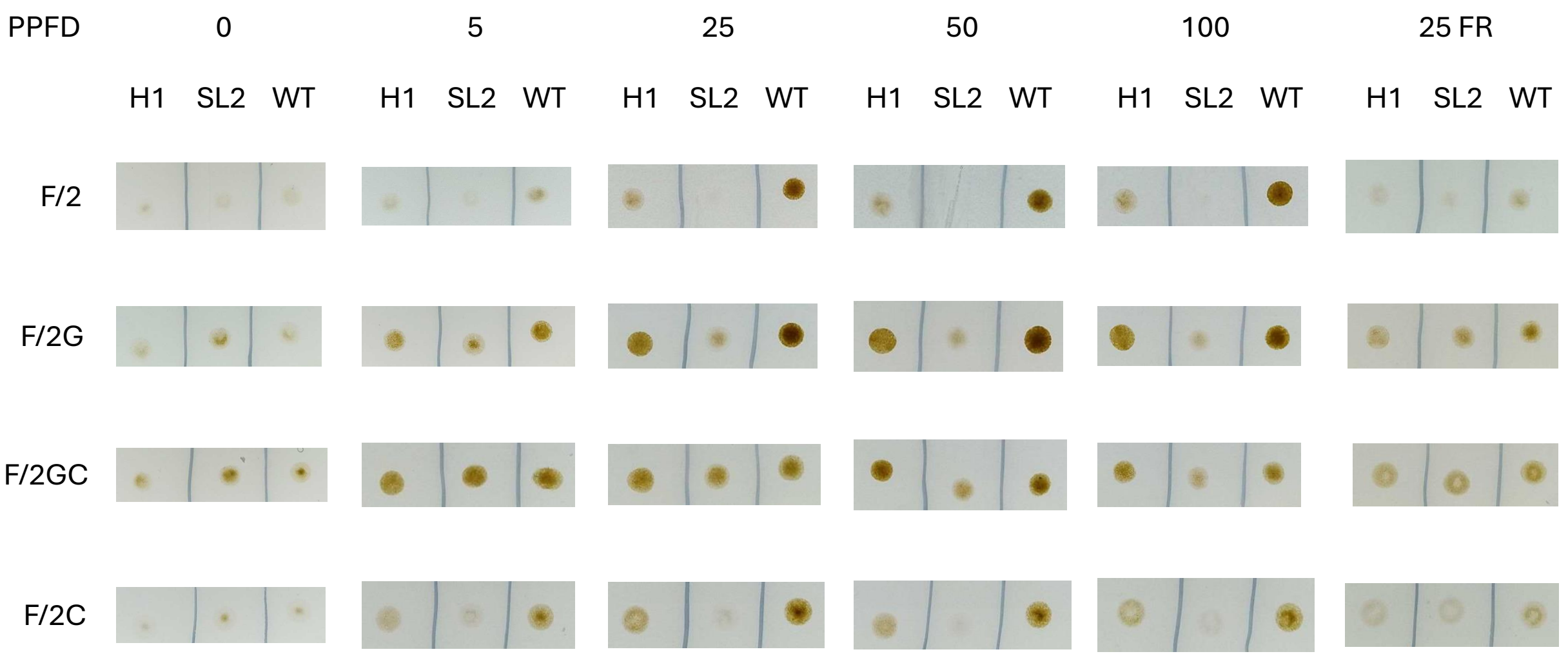







Chlorophyll per cell
Chl conc per cell (ug chl/cell)
6e-06
4e-06
2e-06
ΔpsbI
ΔpsbE
WT
Strain
Strain
ΔpsbI
ΔpsbE
WT

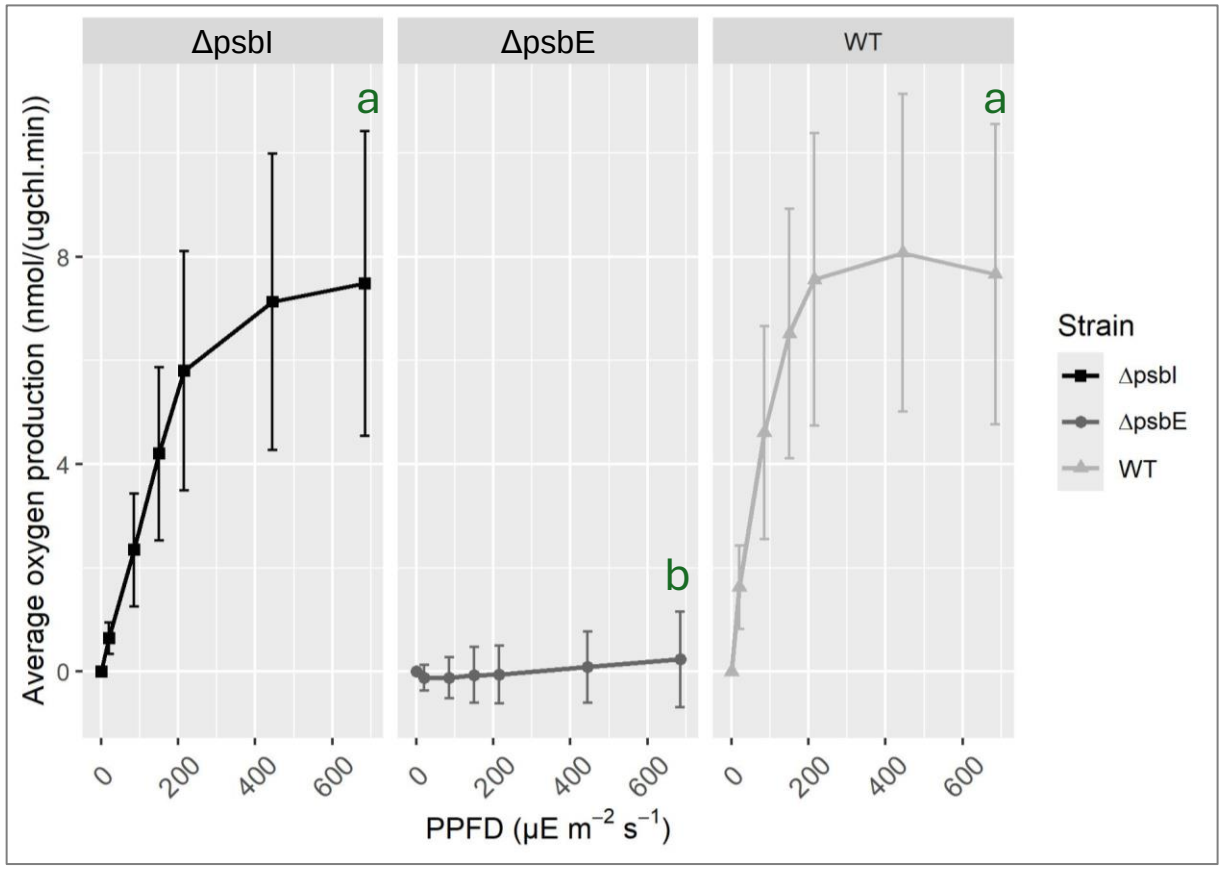
3. **Results** – Growth on plate



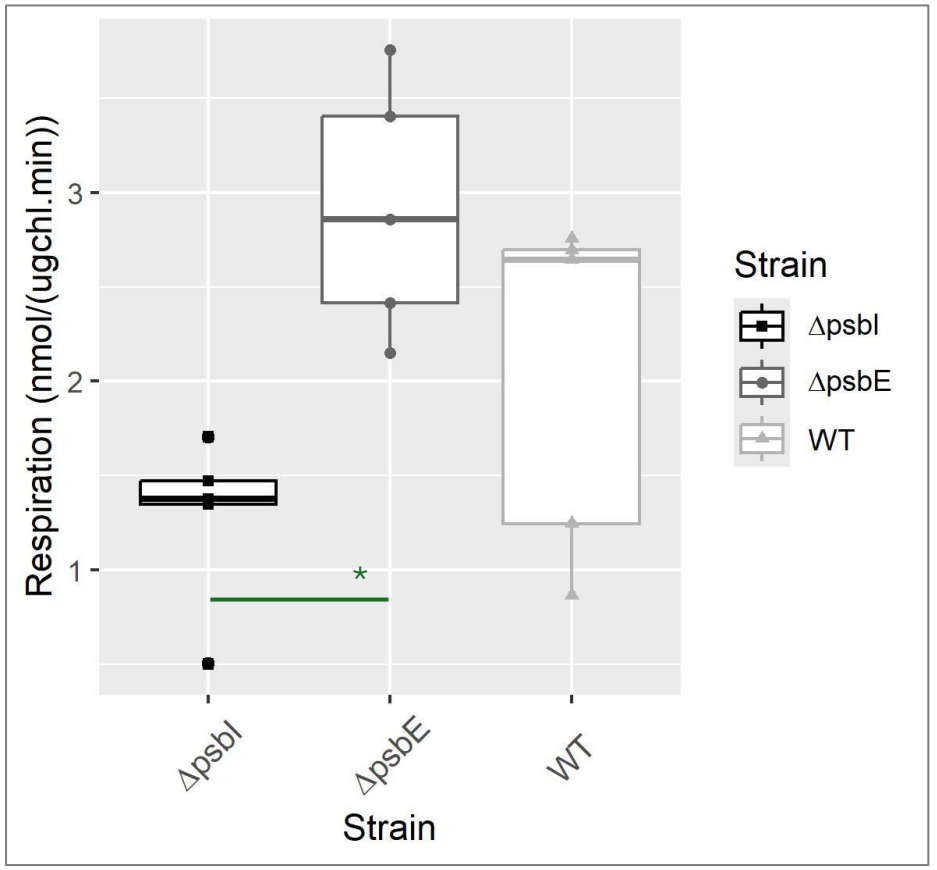
3. **Results** – Oxygen production and uptake

Oxygen production

(3 min light steps)



Respiration

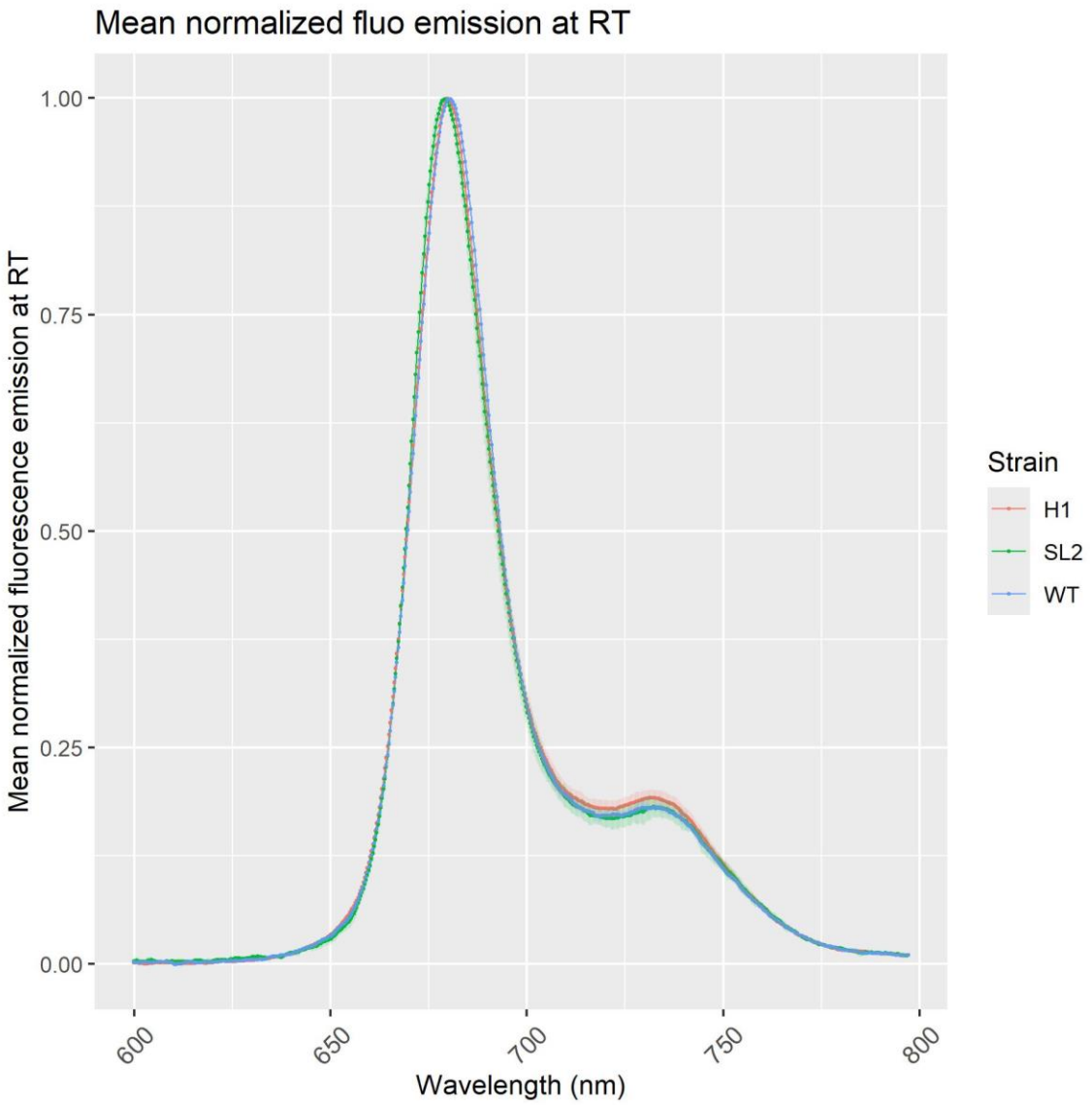


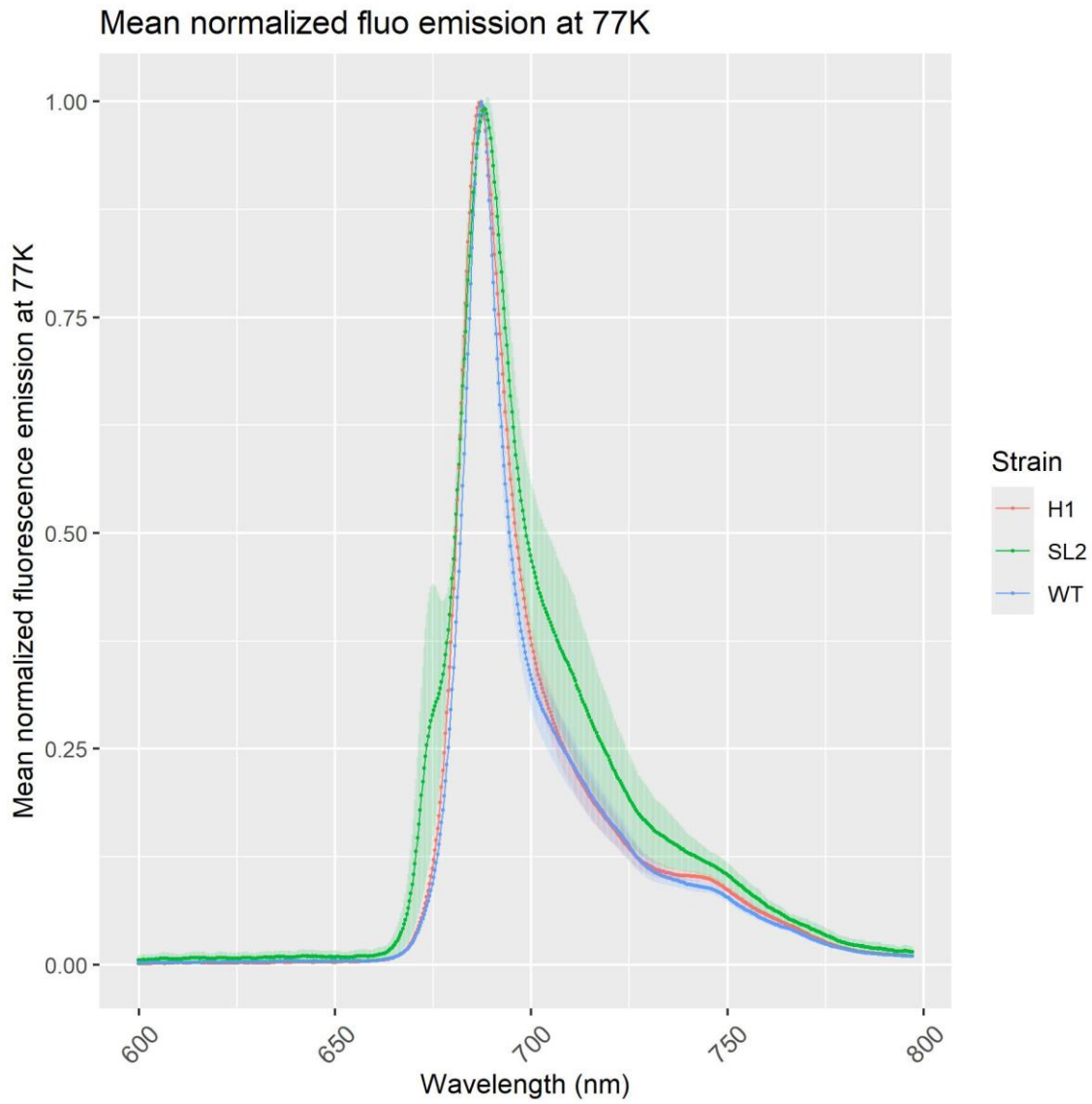
→ Non functional PSII in ΔpsbE strain

→ Similar O_2 production in ΔpsbI and WT

→ Slightly lower respiration for ΔpsbI ?

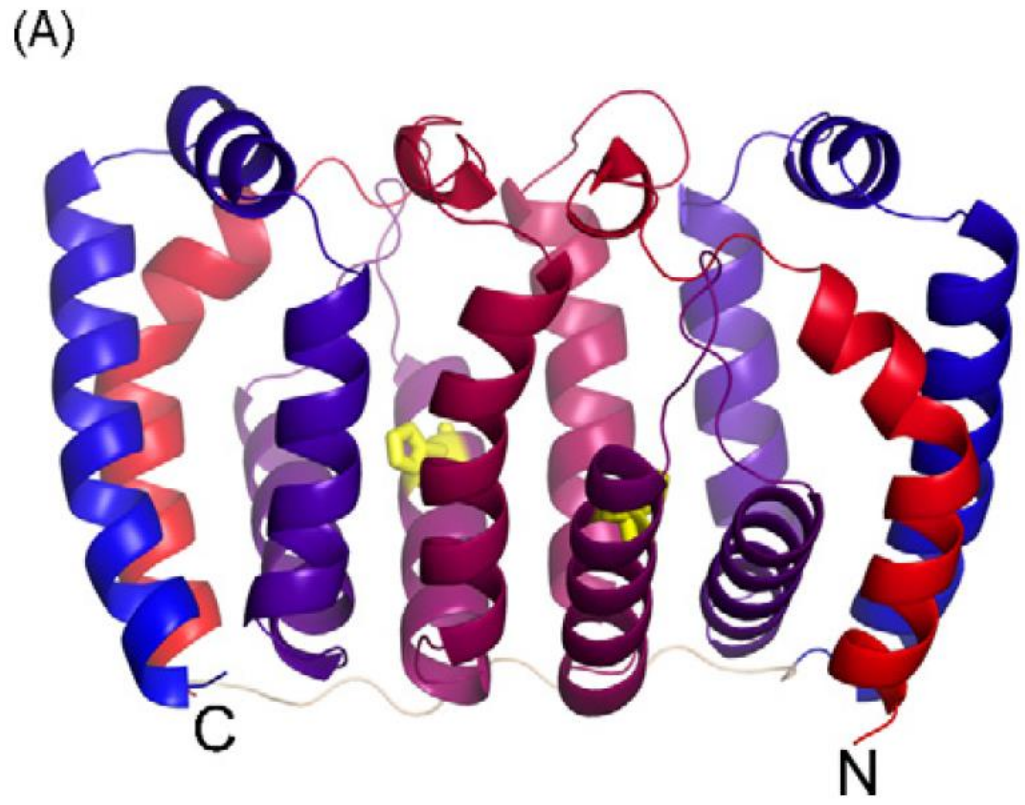
3. **Results** – Fluorescence emission

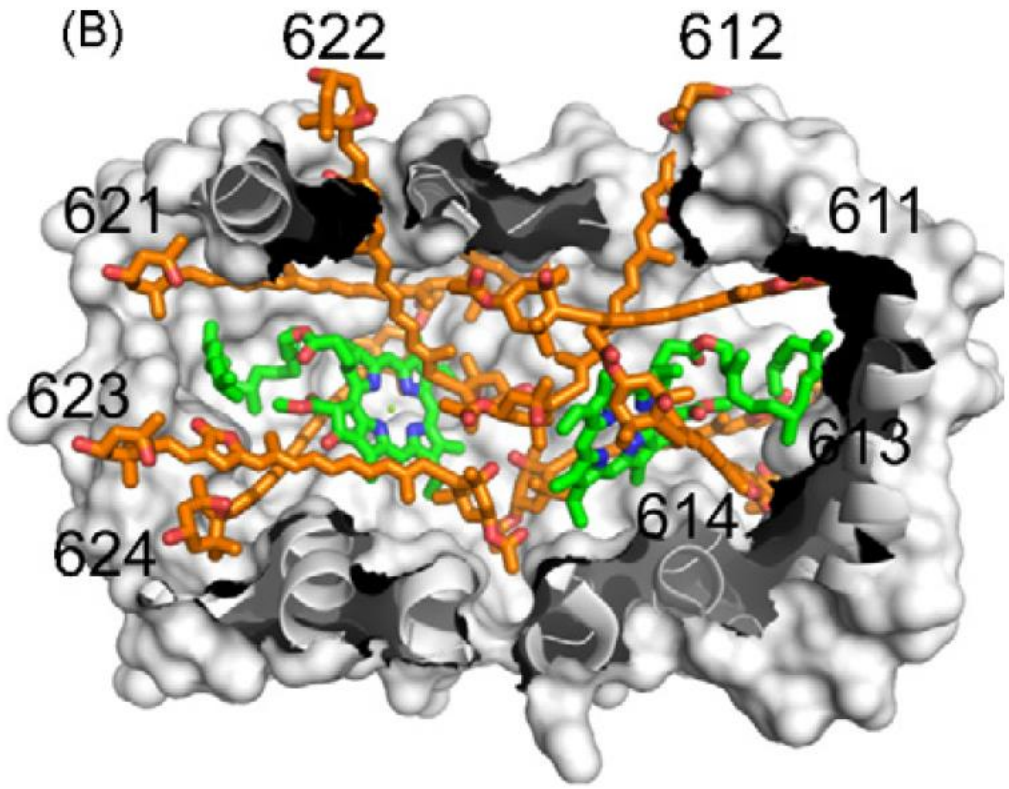




1. Introduction

PCP structure (Schulte 2010)

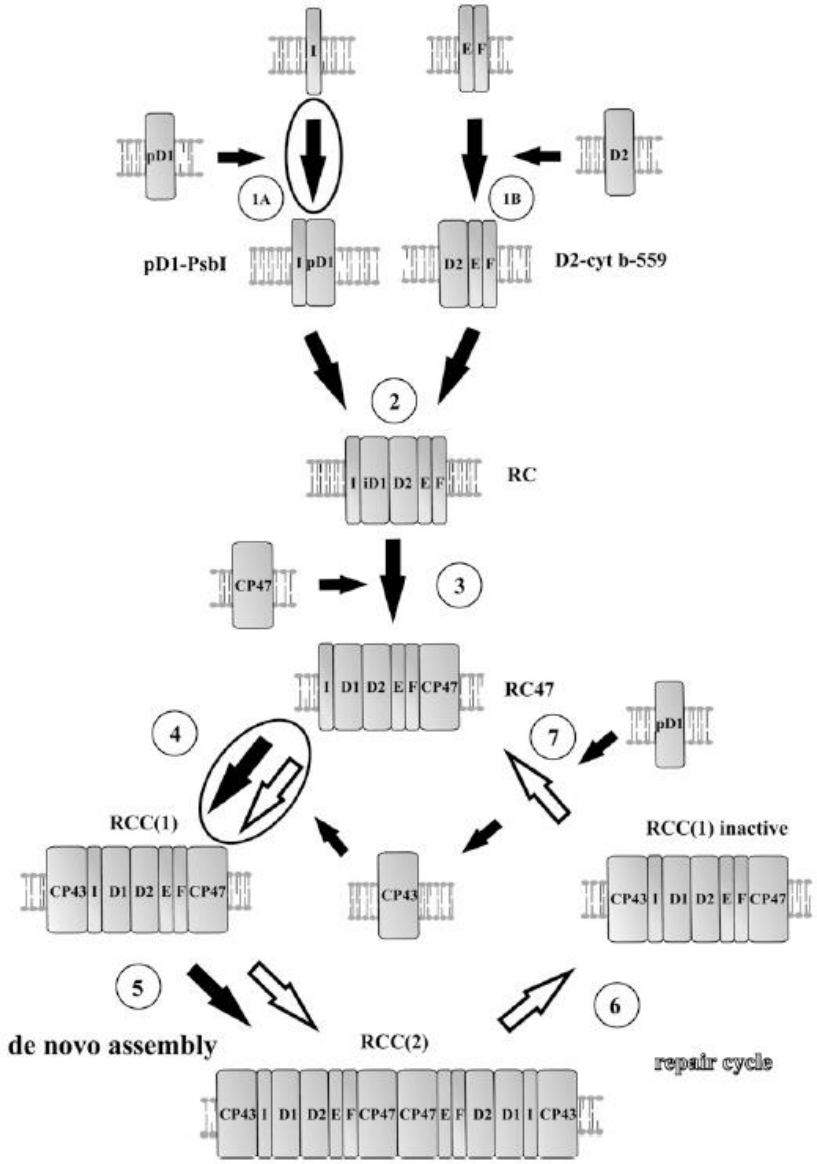




4 peridinin (orange) for 1 chl a (green)

1. Introduction – role of psbI and psbE in chlamy

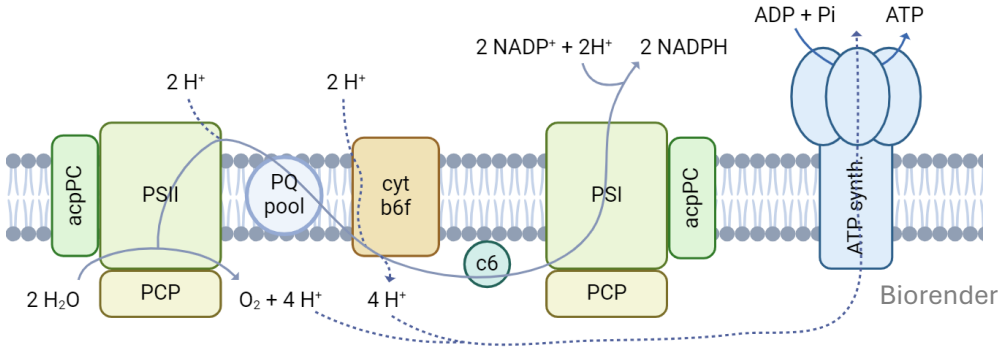
Dobakova 2007



5. Prospectives

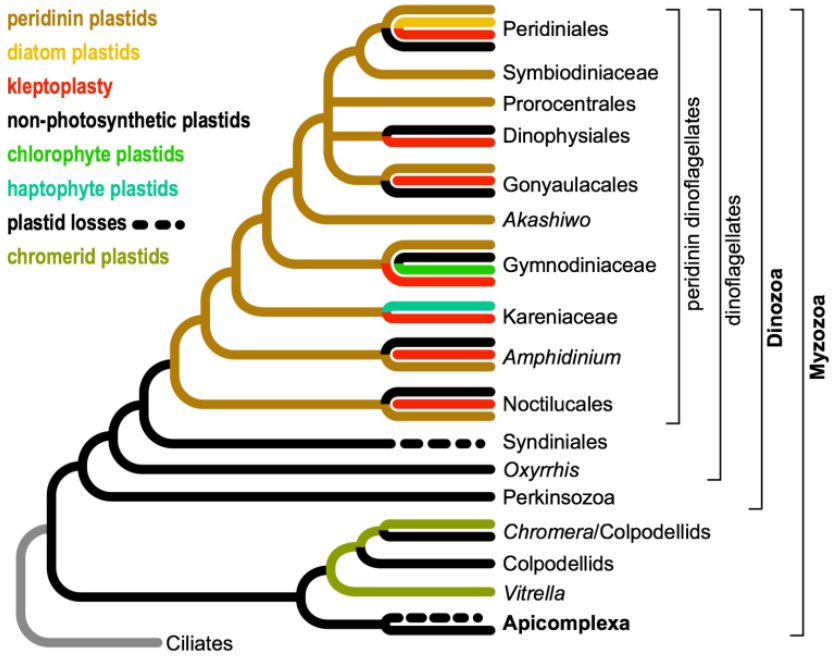
Photosynthesis in *Symbiodinium*

- Pigment composition
- NPQ
- PSII structure
- Cell ultrastructure



Loss of photosynthesis in dinoflagellates

- Strains derivation monitoring
- Analysis of mutant strains appearance



Waller 2017 (6)