BIOCATALYSIS IN GREEN AND BLUE: SYNECHOCOCCUS PCC11901 AS A NEW WORKHORSE

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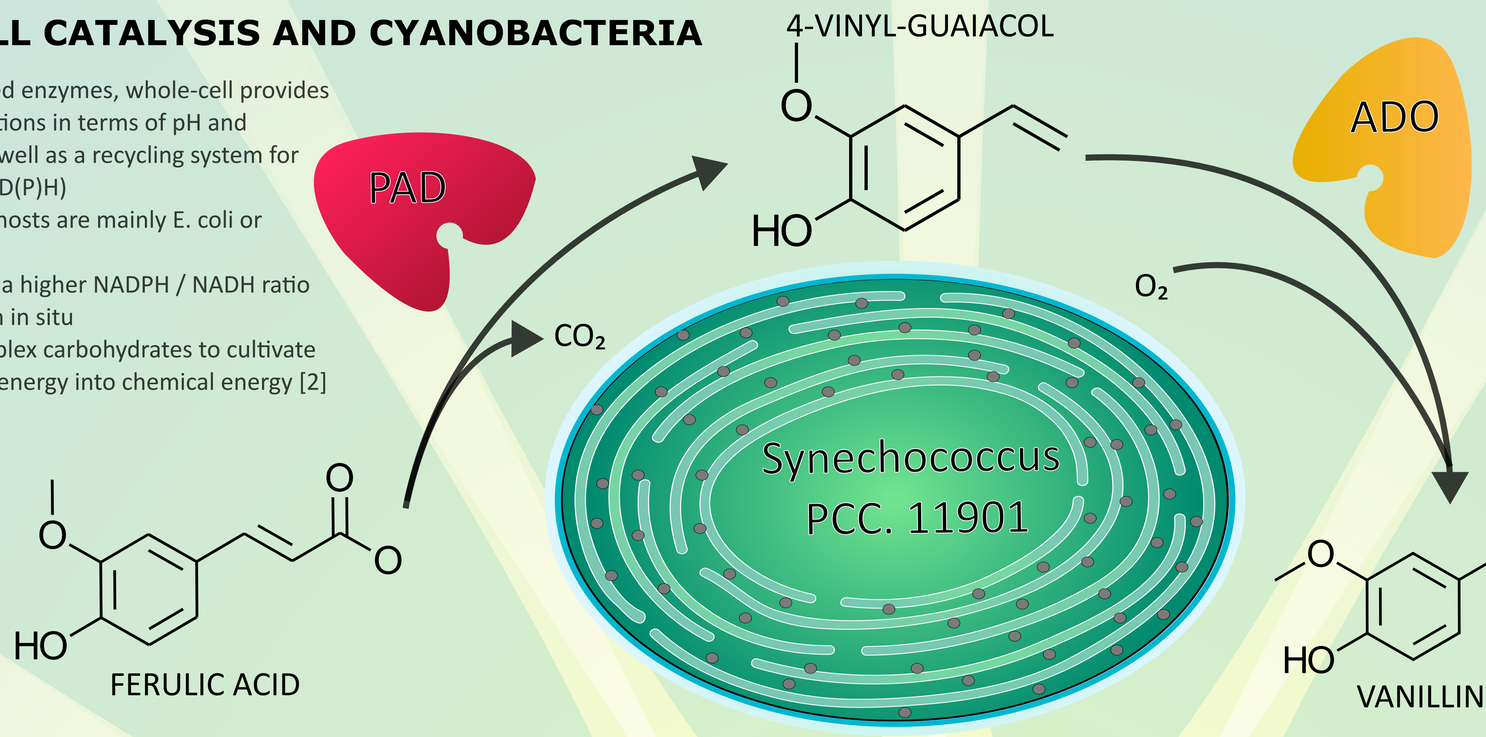
WHOLE-CELL CATALYSIS AND CYANOBACTERIA

- Compared to isolated enzymes, whole-cell provides stable reaction conditions in terms of pH and osmotic pressure, as well as a recycling system for co-factors (ATP or NAD(P)H)
- Commercially used hosts are mainly E. coli or yeasts [1]
- Cyanobacteria have a higher NADPH / NADH ratio
- Produce free oxygen in situ
- Do not require complex carbohydrates to cultivate
- Can transform light energy into chemical energy [2]



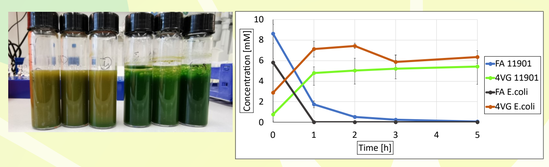
PROBLEM: **Cyanobacteria grow slow and have a low maximal cell density**

SOLUTION: **Synechococcus elongatus PCC11901**

In this proof-of-concept work, we wanted to test, whether Synechococcus elongatus PCC 11901 is a suitable candidate for whole-cell catalysis, as it has been described as a fast growing strain, with the highest maximal cell density of any cyanobacterium [3]. A phenolic acid decarboxylase (PAD) is used to convert ferulic acid (FA), a waste product from lignin degradation into 4-vinyl-guaiacol (4VG), a value-added flavor-compound [4].

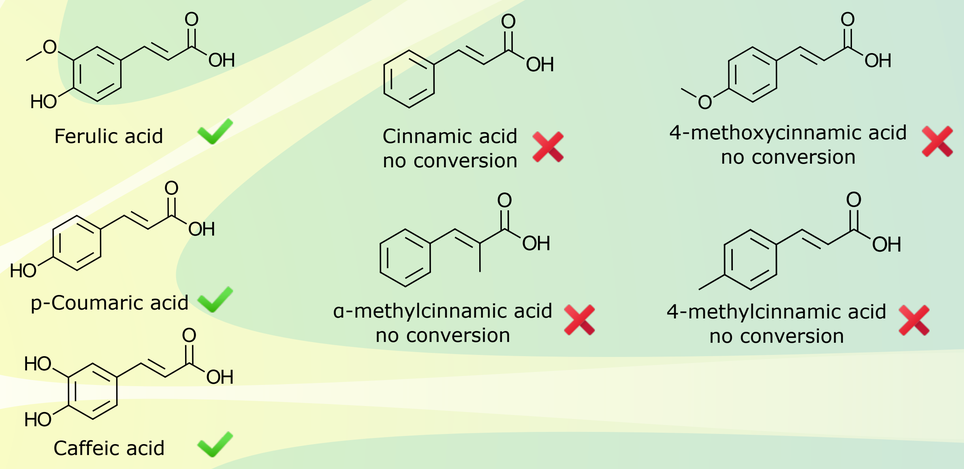
PHENOLIC ACID DECARBOXYLASE

We inserted a phenolic acid decarboxylase into the fadA gene with a kanamycin resistance cassette and lacI expression cassette. We see good expression after induction with 1mM IPTG and only basal expression without IPTG.
Product-toxicity halts conversion of ferulic acid (FA) into 4-vinyl-guaiacol (4VG), but with an organic phase as overlay for in situ product removal, full conversion of 10 mM ferulic acid was possible after 5h, comparable to *E. coli* at the same cell density.
We observed in our set-up, that the oxygen production of cyanobacteria depends on light and the available carbon-source (CO_2 or carbonate). The measurement of oxygen could so be used as an indicator for product formation, albeit the product strongly suppresses photosynthesis.



LEFT: PCC11901 fadA::pad with and without an DINP overlay, 24h after adding 10 mM Ferulic acid RIGHT: Comparison of Syn 11901 fadA::pad to E.coli pET28_PAD

Substrate Scope of PAD in *Synechococcus*



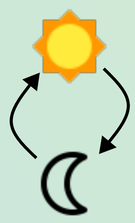
AROMATIC DIOXYGENASE

The aromatic dioxygenase should convert 4-vinyl guaiacol into Vanillin, unfortunately, our attempts to insert the gene into the genome of *Synechococcus PCC.11901* were not fruitful. A series of variations in an attempt to increase the transformation efficiency showed no effect. Efforts included:
- Change the insertion site and size of fragment to integrate
- Exploiting the circadian clock
- Using 4 vinyl-guaiacol as additional selection marker

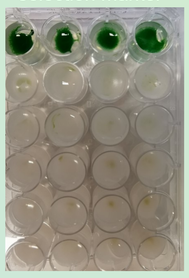
Genetics

Constructs
Psba::ado
ARTO:ado
COX:ado
Psba::ado -lacI
ARTO:ado -lacI
COX:ado –lacI
psbA:: mapADO
ARTO:: mapADO
COX::mapADO

Exploit day/night rythm

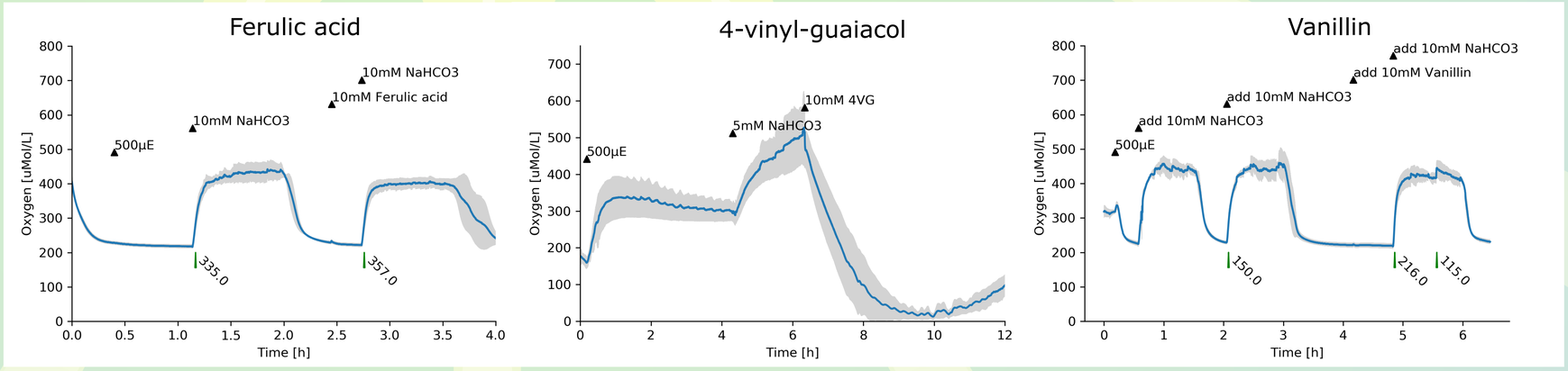


Using substrate as a selection marker



TOXICITY ASSAY VIA OXYGEN MEASURMENT

The oxygen evolution rate (OER) of cyanobacteria depends on light intensity as well as available inorganic carbon [4]. This means, at high light and depleted carbon in the medium, a change in carbon availability and photosynthetic activity can be measured by dissolved oxygen. Here, we used this, to test the effect of our substrate (ferulic acid), intermediate (4 Vinyl-guaiacol) and product (Vanillin) on the oxygen evolution rate and thus on the "health" of our culture. First we add a certain amount of sodium bicarbonate (5 or 10 mM), to see the status quo of our culture, than we added 10 mM of the compound of interest and again add carbonate to see the difference. Our results imply, that while ferulic acid and Vanillin have little to no effect on OER, 4-Vinyl-guaiacol immediately brings photosynthetic oxygenation to a halt.



Synechococcus PCC11901 WT cells were grown for 48h in MAD2 medium (salt-water medium for high cell density) at 500µE, 1% CO_2 , 150rpm, than washed 2 times in swee-water medium with 100mM HEPES (BG11-HEPES) to a final cell density of ~20 gCDW/L. For oxygen-measurement, Pyroscience firesting (Aachen, Germany) was used. Numbers under the curve represent the oxygen evolution rate in nmol O_2/h/µg chlorophyll a.

SUMMARY & OUTLOOK

- *Synechococcus PCC11901* can be used as a host for whole-cell catalysis and can convert 10 mM ferulic acid into 4-vinyl-guaiacol
- Product-toxicity can be circumvented by in situ product-removal with an organic phase & aromatic dioxygenases cannot be insertated into our host genome
- Carbonate induces high oxygen production under high light environments and can be used to monitor toxicity online and to optimize reaction conditions

References and Acknowledgments

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