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Whole-cell catalysis in green and blue: Synechococcus PCC11901 as a new workhorse?

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PURPOSE OF THE ABSTRACT

BIOCATALYSIS IN GREEN AND BLUE: SYNECHOCOCCUS PCC11901 AS A NEW WORKHORSE?
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Can we exploit Photosynthesis for Whole-cell-catalysis? In oxygenic, photoautotrophic organisms, light energy provides redox-energy and oxygen accumulates. An ideal “reaction-vessel” for oxygenating enzyme-catalyzed reactions. Fast co-factor recycling without the need for sacrificial co-substrates and thus for whole-cell catalysis1.
Cyanobacteria, ancient photoautotrophic prokaryotes, have gained interest by the biotechnology community for good reasons. They grow faster than other photosynthetic active organisms and their comparatively simple genetic built render them accessible to genetic engineering. Despite their advantages, Cyanobacteria lag behind more commonly commonly used, heterotrophic microbes, as they grow more slowly and less densely and reliable high expression of enzymes is often difficult to achieve2.
In this project, we wanted establish whole-cell catalysis in a cyanobacterial strain, that could overcome some of the current drawbacks of cyanobacteria by implementing an apt two-step enzymatic reaction.
We planned to test a two-step enzymatic reaction from ferulic acid to vanillin in the newly discovered Synechococcus PCC 11091. This strain is one of the fastest cyanobacteria in terms of growth and reach the highest cell density ever measured in cyanobacteria grown in a shake flask3. The enzymes of interest turn ferulic acid into 4-vinylguaicol and release one molecule of CO2 and in the second step react with O2 to Vanillin, releasing formaldehyde. Our hypothesis was, that by removing CO2 and providing O2, the photosynthetic metabolism of Synechococcus PCC11901 boosts the reaction efficiency4.
While the first enzyme of this mini-cascade (Phenolic Acid Decarboxylase (PAD)) was stably inserted into the hosts genome and showed high reaction rates, the second enzyme (Aromatic dioxygenase (ADO)) could not be stably inserted into the hosts genome. We also tested a newly discovered enzyme, called mapADO (not published). This enzyme showed a higher activity and better expression in E. coli. but continued to evade our attempts to be inserted into the cyanobacterium's genome.
As this idea approached a dead-end, we tried a “work-around”. While E. coli expresses our enzyme of interest, we added Cyanobacteria to the reaction, in hope to improve yield by shifting the reaction equilibrium. In addition to substrate and product concentration, we also monitor oxygen content and chlorophyll content. With this, we hope to find the best conditions for our reactions to occur and prove the additional benefits, Synechococcus PCC11901 has on whole cell catalysis.

FIGURES

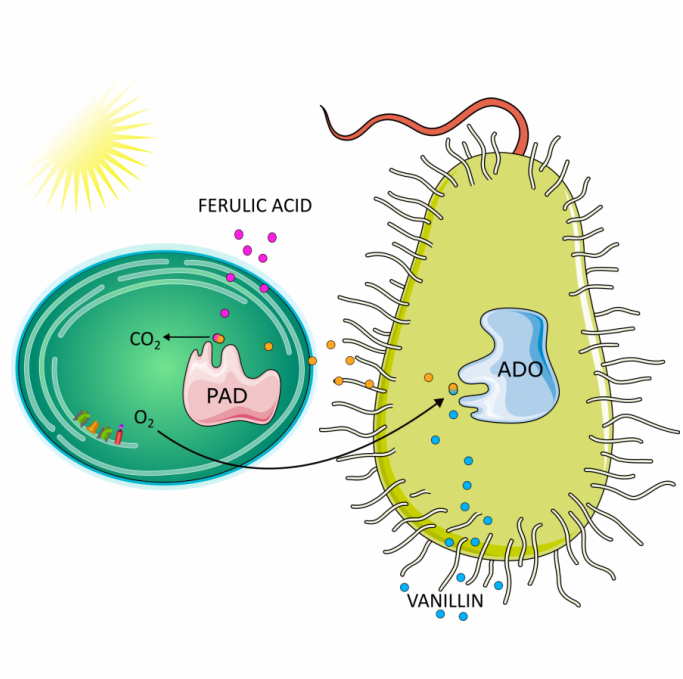

FIGURE 1

Schematic of two-whole-cell catalysis

E. coli (right) expresses the two necessary enzymes (PAD and ADO) to turn Ferulic acid into 4-Vinylguaicol and subsequently into Vanillin. Synechococcus elongatus PCC11901 (left) consumes the produced CO2 and produced CO2 and produces O2 to shift the reac

FIGURE 2

KEYWORDS

Cyanobacteria | Whole-cell catalysis | Oxygenating Enzymes

BIBLIOGRAPHY