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A high-throughput characterization strategy of a genetic toolbox for the improvement of cyanobacteria as hosts for whole-cell biocatalysis

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PURPOSE OF THE ABSTRACT

Cyanobacteria are promising and, quite literally, green candidates for microbial production platforms. Their photoautotrophic metabolism enables a sustainable production system by depending solely on water, carbon dioxide, and sunlight. They have also been shown to be valuable in whole-cell biocatalysis, producing large amounts of the energy-rich cofactor NADPH and oxygen in situ, which is of interest for the functionality of numerous industrially applied enzymes [1].

Despite these significant advantages, applicability was limited so far by remaining challenges. One of the biggest challenges is still to obtain sufficient expression levels of recombinantly expressed proteins within the cyanobacterial host. Furthermore, their photoautotrophic cultivation limits high-throughput screening possibilities, which also slows down progress within the field. To push cyanobacteria further to its limits, it is, therefore, necessary to improve the capacities and understanding of the host system.

Within this work, we aim to tackle these challenges and improve cyanobacteria as hosts for biocatalysis. (i) We built a genetic toolbox to improve enzyme yields within the host. (ii) We developed a characterization strategy to analyze the genetic regulatory elements in a one-pot approach. To exemplify the strategy, we applied three enzymes to improve their performance in cyanobacteria. As enzymes, we investigated a ketoreductase (LfSDR1M50) [2], an enoate reductase (YqjM) [3], and a Baeyer-Villiger monooxygenase (CHMOAcineto) [4].

The genetic toolbox is built up of 12 different promoters and 21 ribosome binding sites, which were designed to be an extension of the cyanobacterial MoClo Kit, CyanoGate [5]. To screen and characterize all expression constructs in a one-pot manner, we fused a GFP tag C-terminally to the investigated enzymes. Like this, the GOI-specific expression libraries could be analyzed for their expression levels according to their fluorescence and, by flow cytometry, sorted from low-expression strains to high-expression strains. The genetic context of the sorted cells was then analyzed by deep sequencing.

As a result, we got an in-depth characterization of the individual regulatory elements under different genetic contexts (LfSDR1M50-GFP, YqjM-GFP and CHMOAcineto-GFP). A broad range of different expression levels could be achieved. Here, the promoter, as well as the RBS, have a significant impact. Interestingly, by only varying the RBS, we reach a similar scope of various expression levels compared to solely varying the promoter. With this study, we therefore emphasize that RBSs are often underrated when designing genetic constructs despite this significant impact. However, as demonstrated previously [6], we see that the efficiency of an RBS varies strongly among different genetic contexts (GOIs), making it more challenging to predict a priori. Therefore, this study aims to bring further insights into RBS performance and underline the current shortage of understanding.

To summarize, these results underline the untapped potential and the importance of further improving expression systems in cyanobacteria. Here, with our study, we present a promising approach to exploit further potential to find improved expression strains and whole-cell biocatalysts.

FIGURES

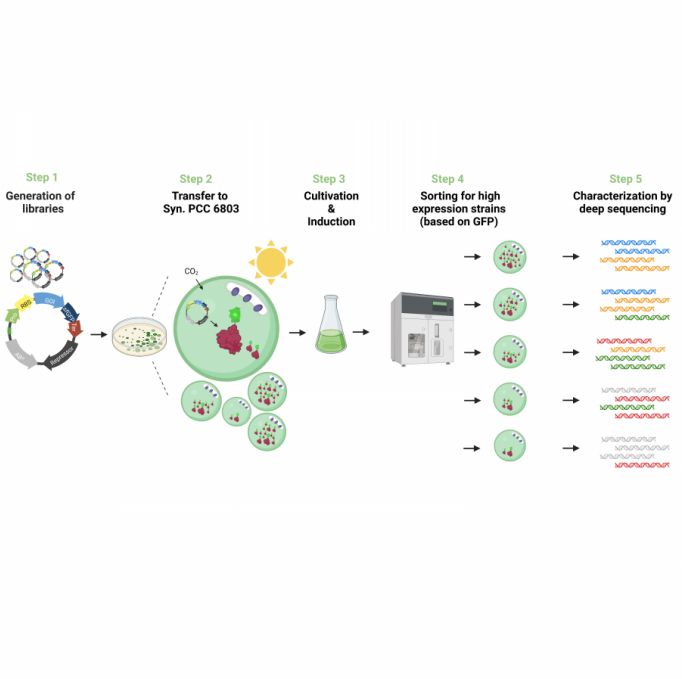

FIGURE 1

Characterization strategy of a genetic toolbox to improve gene expression in cyanobacteria

An expression library with different combinations of RBSs and promoters is generated. A C-terminal GFP fusion tag allows the screening and analysis of the expression of a gene of interest (GOI) by FACS and subsequent deep sequencing.

FIGURE 2

KEYWORDS

cyanobacteria | genetic toolbox | high-throughput | whole-cell biocatalysis

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