Production of Valuable Carbon Containing Products with a new Module-Based Vector System in Cyanobacteria

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Abstract

Regarding climate change, the reduction of CO_2 emissions became one of the major topics in the last few years. On the one hand, photosynthetically active organisms, like cyanobacteria, offer a great possibility to reduce CO_2 emissions by using photosynthesis and to synthesize various biofuels and chemicals by genetic engineering. On the other hand, this strategy leads to a reduction of the dependency on petrol-based fuels in our society. Gao *et al.* [1, 2] showed enhanced ethanol production up to 5.5 $g \cdot l^{-1}$ by using the genetically engineered cyanobacteria *Synechocystis sp.* PCC6803. Based on these improvements, strategies for obtaining higher cell densities and further enhanced ethanol yield were developed. Therefore, a new module-based expression vector system with the possibility for easy exchange of DNA fragments, such as homologous integration sites, promoters and genes, was designed and is presented. For ethanol production, the genes encoding alcohol dehydrogenase and pyruvate decarboxylase will be integrated into the cyanobacterial genome. Constitutive as well as inducible promoters will be tested for maximum ethanol production in *Synechocystis sp.* PCC6803 in a single or a two-step cultivation process. Strategies and gene constructs for high ethanol production in *Synechocystis sp.* PCC6803 are presented.

Background

The cyanobacteria *Synechocystis sp.* PCC6803 – *Syn* is a unicellular, non-nitrogen fixing and naturally competent fresh water organism, which serves as model organism for studying *e.g.* photosynthesis. In *Syn*, different chemical compounds, like fatty acids or different organic acids and alcohols, can be synthesized [2]. However, ethanol, used as non-petrol based fuel, is not produced naturally by *Syn*. Therefore, the overexpression of the two genes pyruvate decarboxylase – *pdc* (heterologous, *Zymomonas mobilis*) and alcohol dehydrogenase – *adh* (endogenous), via genomic double integration, were the most productive, yet [1].

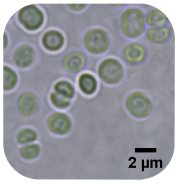

Fig. 1: Synechocystis sp. *PCC 6803 Zoom x 1000*

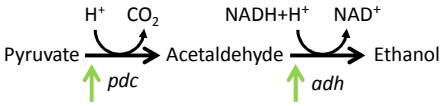

Fig. 2: Ethanol producing pathway with involved enzymes

Further enhanced ethanol yield may be reached with a stronger promoter. For this purpose, the at least four fold stronger promoters P_{cpc560} (light activated) and P_{trc1O} (IPTG induced) are very promising [3, 4].

Strategies

To pursue different production strategies, new module-based expression cassettes, based on the pEERM3 KM vector [5], were designed.

Inducible Two-Stage Process

+ four fold stronger P_{trc1O} promoter
+ fully controlled expression of genes
+ shift of metabolic stress into a separate production stage
+ prevention of putative product inhibition of growth

- cells need to have energy reserves for ethanol synthesis

Constitutive Single-Stage Process

+ four fold stronger P_{cpc560} promoter
+ ethanol synthesis during the whole process time

- constitutive expression may inhibit growth
- possible growth inhibition by ethanol
- P_{cpc560} transcription rate is inversely proportional to cell density

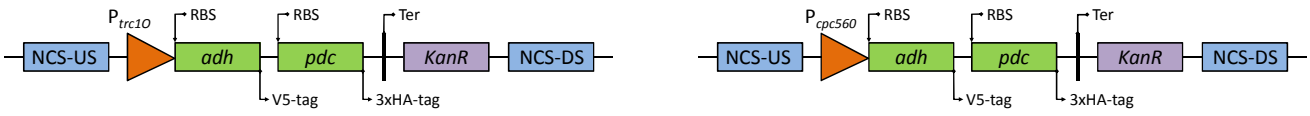

Fig. 3: Module-based expression cassettes for different cultivation strategies; NCS…neutral cloning site; US…upstream; DS…downstream; KanR…kanamycin resistance cassette; RBS…ribosomal binding site; P_{xxx}…promoter; Ter…terminator

Outlook

At present, ethanol yields with *Syn* were far too low for commercial exploitation. As double integration of the same construct led to a nearly five fold higher ethanol yield [1], a four fold stronger promoter is expected to increase ethanol yield even further. To compare both production strategies, mutants will be characterized and ethanol yield will be determined. In further approaches, knock-out of alternative pyruvate consuming pathways, e.g. polyhydroxybutyrate synthesis, with the expression constructs are planned.

References: [1] Z. Gao *et al.* , "Photosynthetic production of ethanol from carbon dioxide in genetically engineered cyanobacteria," Energy Environ. Sci., vol. 5, no. 12, pp. 9857–9865, 2012.
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[3] H. H. Huang *et al.*, "Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology," Nucleic Acids Res., vol. 38, no. 8, pp. 2577–2593, 2010.
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[5] E. Englund *et al.*, "Metabolic Engineering of Synechocystis sp. PCC 6803 for Production of the Plant Diterpenoid Manoyl Oxide," *ACS Synth. Biol.*, vol. 4, no. 12, pp. 1270–1278, 2015.

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