From Glycogen to Starch – Cyanobacteria as a Production System for Carbon Containing Products

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Abstract

Nearly 29 gigatons of CO_2 are exhaled by humans every year, which influence the Earth´s climate significantly. A decrease of this amount combined with the production of valuable products sounds like a futuristic idea. But in fact, photosynthesis, *e.g.* performed by cyanobacteria, uses CO_2 to synthesize carbon-containing products. For this reason, cyanobacteria provide a promising approach to convert CO_2 into valuable products [1]. Starch could be such a product with regard to its versatile application in aqua feed, the paper industry, processed food, animal and pet food as well as pharmaceuticals and cosmetics. Unfortunately, starch is not naturally produced by cyanobacteria. Here, glycogen is synthetized, which consists of a higher linkage grade of α1.4 and α1.6 glucose chains compared to starch [2]. The starch production will be performed in two steps: In a first step, the amount of glycogen precursors will be increased by overexpression of four genes, which are part of the glycogen synthesis pathway. In a second step, the endogenous branching enzyme will be exchanged by a eukaryotic one (*Arabidopsis thaliana*) to convert the produced glycogen into starch. All expression constructs will be integrated into the genome of *Synechocystis sp.* PCC 6803 (*Syn*) via homologous recombination. Afterwards, the genomic integration, as well as protein production will be analyzed next to the determination of the linkage grade of the synthesized product.

Background

Synechocystis sp. PCC 6803 is a unicellular, polyploid, non-nitrogen fixing cyanobacteria, which is attractive as a photo-autotrophic factory to produce valuable products by the consumption of CO_2 and light [1].

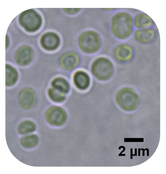


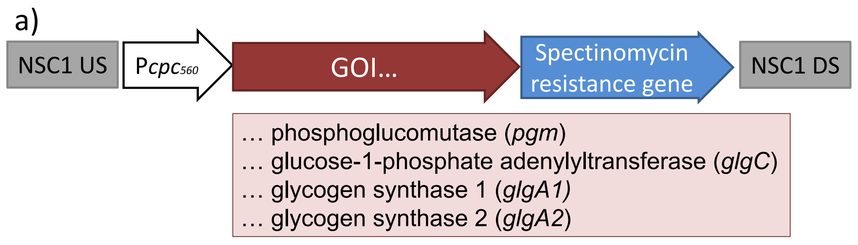
Fig. 1: Synechocystis sp. *PCC 6803 Zoom x 1000*

Status

1) Cloning of Expression Plasmids for…

… overexpression of glycogen precursors

a)



… convertion into starch

b)

Fig. 2: Expression DNA a) constructs with genes, which are part of the glycogen synthesis pathway to increase the amount of glycogen, b) construct with the eukaryotic branching enzyme to generate starch like branches. GOI…gene of interest (either glgA1, glgA2, glgC *or* pgm*), NSC1US/DS…neutral site upstream/downstream [3], SYN US/DS…upstream/downstream region of the natural glycogen branching enzyme.*

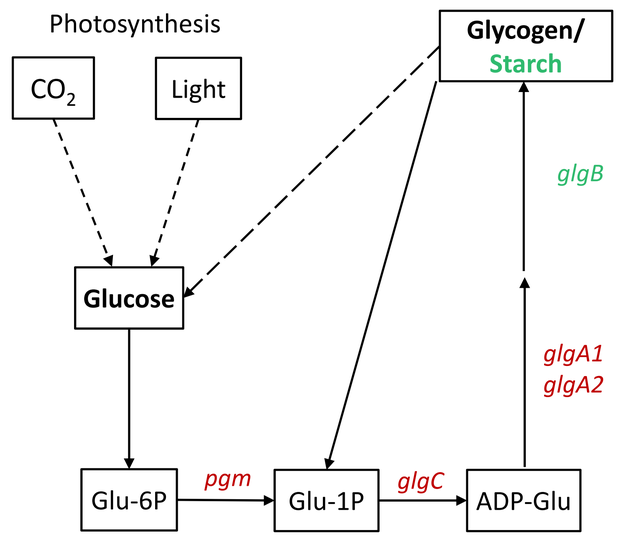


Fig. 3: Part of the glycogen metabolism of Synechocystis sp. *PCC 6803; green: exchange of the natural glycogen branching enzyme; red: genes which will be overexpressed [4].*

Workflow

2) Transformation

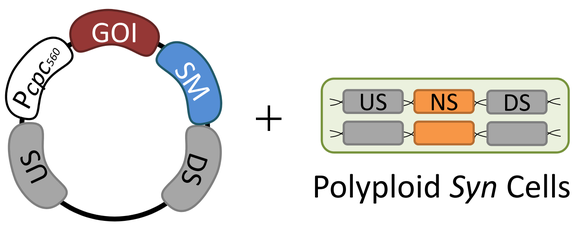


Fig. 4: Diagram of the transgene integration into the Syn *genome via homologous recombination. GOI…gene of interest (either* glgA1, glgA2, glgC *or* pgm*), US/DS…neutral site upstream/downstream, SM…selection marker, NS…neutral site.*

3) Segregation

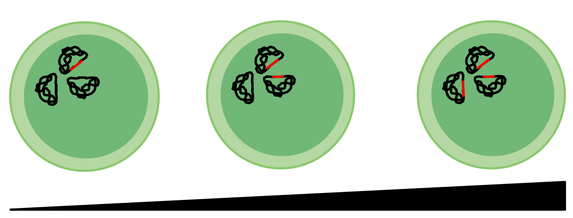


Fig. 5: Schematic intregration of the transgene into all genomes of Syn *due to increasing concentrations of antibiotics.*

4) Verification at …

… DNA level: multiplex colony PCR

… protein level: immunological assay

… linkage grade: glycogen/starch

Outlook

Future studies will focus on increasing the yield of starch in transgenic cyanobacteria. Combining all four genes, which are part of the glycogen synthesis pathway, on one expression plasmid and transform it into the genome will be one possible approach. The knock out of alternative glucose consuming pathways as the polyhydroxybutyrate metabolism will be an other alternative technique [4]. Moreover, the strategy of first increasing the cell density and afterwards inducing the starch production due to the IPTG-induced promotor P_{trc1O} presents yet another alternative [3].

References:

[1] D. Lui, H. B. Pakrasi (2018), "Exploring Native Genetic Elements as Plug-In Tools for Synthetic Biology in the Cyanobacterium Synechocystis sp. PCC 6803", Microbial Cell Factories, Vol. 17, No. 1, p.1.

[2] S. Yoo, B. Lee, Y. Moon, M. Spalding, J. Jane (2014), "Glycogen Synthase Isoforms in Synechocystis sp. PCC6803: Identification of Different Roles to Produce Glycogen by Targeted Mutagenesis" , PLoS ONE, Vol. 9, No. 3.

[3] A. H. Ng, B. M. Berla, H. B. Pakrasi (2015), "Fine-Tuning of Photoautotrophic Protein Production by Combining Promoters and Neutral Sites in the Cyanobacterium Synechocystis sp. Strain PCC 6803", App. Environ. Microbiol., Vol. 81, No. 19, p. 6857 - 6863.

[4] C. Gonzalez-Fernandez, M. Ballesteros (2012), "Linking Microalgae and Cyanobacteria Culture Conditions and Key-Enzymes for Carbohydrate Accumulation. Biotechnology Advances", Vol. 30, No. 6, p. 1655 - 1661.

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