Genetic engineering to improve poly-β-hydroxybutyrate (PHB) production by *Synechocystis* PCC6803 in photoautotrophic conditions

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Serious environmental concerns like safe disposal, solid waste management, and plastic waste incineration justify the production of materials like green polymers/biodegradable plastics/bioplastics utilizing renewable resources.

The biosynthesis of PHB directly from carbon dioxide (CO_2), is a sustainable alternative for non-renewable, petroleum-based polymer production. The conversion of CO_2 implies a reduction of greenhouse gas emissions. *Synechocystis* PCC6803 can store PHB using CO_2 as a carbon source, i.e., through an photoautotrophic conversion.

In this study *Synechocystis* sp. PCC6803 was genetically modified to improve PHB production from CO_2.

The deletion of phosphotransacetylase (*pta*) and acetyl-CoA hydrolase (*ach*) to reduce acetate production suggested an approach to increase PHB accumulation. Moreover the overexpression of phosphoketolase (*xfpk*) was used as strategy to increased acetyl-CoA production and subsequently PHB production. Seven mutant strains were created by single and combined *pta* deletion, *ach* deletion and *xfpk* overexpression.

To the author's knowledge, this work was the first work in which *pta* and *ach* deletion and *xfpk* overexpression was evaluated as strategies to improve PHB accumulation in cyanobacteria.

The mutants strains were tested in photobioreactors, in photoautotrophic conditions (a circadian rhythm: 18h light/6h dark) using BG_{11} medium for cyanobacteria growth, for PHB production.

Only the strains in which *xfpk* was overexpressed reported a higher amount of PHB with respect to the wild type strain. These strains were tested on a different growth medium characterized by a different nitrate concentration to further increase PHB production. Indeed, $BG_{1/2}$ medium was the medium in which nitrate concentration was half the optimal concentration (BG_{11} medium).

We successfully obtained a high amount of PHB, approximately 21% w/w of the dry cell weight, in the strain in which *xfpk* gene was overexpressed and *pta* and *ach* genes were knocked out. In particular, the PHB fraction of this mutant strain reached a value 3-fold higher than the one obtained with the wild type strain.