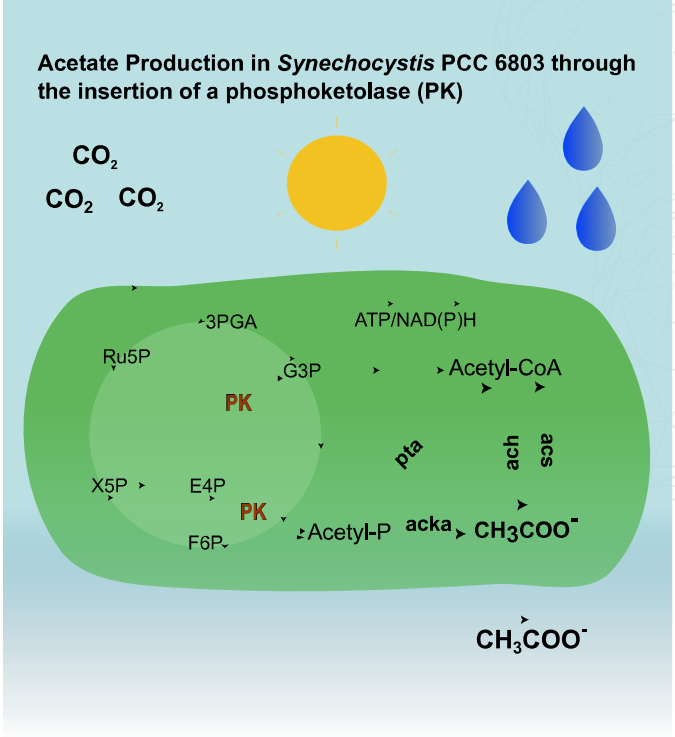
EXPLORING AND INCREASED ACETATE BIOSYNTHESIS IN *SYNECHOCYSTIS* PCC 6803 THROUGH INSERTION OF A HETEROLOGOUS PHOSPHOKETOLASE AND OVEREXPRESSING PHOSPHOTRANSACETYLASE

Key message

The study successfully enhanced acetate production in *Synechocystis* PCC 6803 by introducing a heterologous phosphoketolase (PKPa) and overexpressing phosphotransacetylase (Pta), leading to a significant increase in acetate secretion (up to 2.3 g/L) which is 80 folds more than the wild type. Insertion of the phosphoketolase showed 40 times increase while metabolite analysis also showed enhanced Calvin-Benson-Bassham cycle.



Background

Acetate is an important industrial compound produced biologically by various microorganisms, including cyanobacteria. *Synechocystis* PCC 6803 can produce acetate under specific conditions, but only in low amounts. By modifying metabolic pathways, particularly dragging more carbon to acetyl-phosphate (precursor of the acetate), it is possible to improve acetate synthesis and secretion under photosynthetic conditions.

Objective

To enhance acetate production in *Synechocystis* PCC 6803 by inserting a heterologous phosphoketolase (PKPa) and exploring the key enzymes in the acetate production pathway, including knocking out or overexpress phosphotransacetylase (Pta), acetate kinase (AckA), and acetyl-CoA hydrolase (Ach).

Source

Roussou, S., Pan, M., Krömer, J. O., & Lindblad, P. (2025). Exploring and increased acetate biosynthesis in *Synechocystis* PCC 6803 through insertion of a heterologous phosphoketolase and overexpressing phosphotransacetylase. *Metabolic Engineering*, 88, 250–260. https://doi.org/10.1016/j.ymben.2025.01.008

Results

- **Insertion of PKPa** led to a **40-fold** increase in extracellular acetate compared to wild-type strains.
- **Overexpression of Pta** (in combination with PKPa) further increased acetate production to **80 times the wild-type levels**, reaching **2.3 g/L after 14 days**.
- **Metabolomic analysis** showed increased levels of acetyl-phosphate, fructose-1,6-bisphosphate, and erythrose-4-phosphate, indicating enhanced carbon flux.
- **Knocking out key acetate pathway enzymes (Ach, Pta, AckA)** affected acetate levels differently, with Pta being the most influential in acetate production.
- **Acetate secretion appeared efficient**, suggesting an active transport mechanism rather than passive diffusion.

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