Engineering a Biosynthetic Pathway for Photosynthetic Sorbitol Production in Cyanobacteria

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Background

- *Synechococcus sp.* PCC 7002 is a photoautotrophic strain of cyanobacteria.
- PCC 7002 is an excellent cellular chassis due to fast doubling time and ability to tolerate light, salt, and temperature.
- PCC 7002 can be engineered to produce valuable sugar-alcohols like sorbitol, a target chemical for sustainable production.

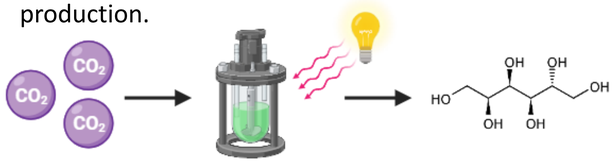


Fig 1. Scaled up process for producing sorbitol using Cyanobacteria

- Currently, PCC 7002 can not effectively produce sorbitol due to promiscuous dehalogenase-like phosphatases which has unwanted side reactions that produce ribose **(Fig 4)**.
- Mannitol-1-phosphatase is a promising candidate since mannitol-1-phosphate and sorbitol-6-phosphate are very similar enantiomers **(Fig 3)**.
- A better BibPase and Aldolase could help reduce Calvin Cycle bottlenecks and increase sorbitol production.

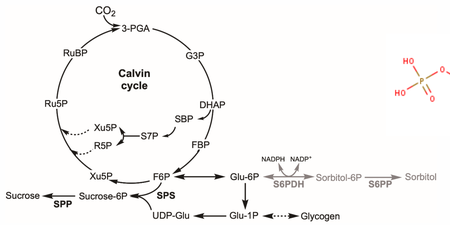


Fig 2. Photosynthetic pathway for sorbitol production in cyanobacteria.

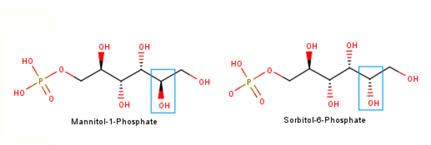


Fig 3. Molecular structures of Mannitol-1-Phosphate
and Sorbitol-6-Phosphate. The blue boxes show the difference in configuration.

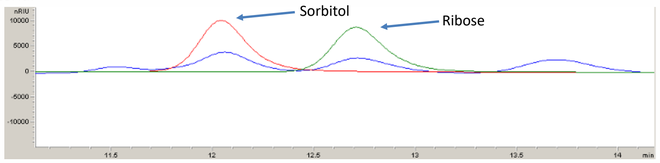


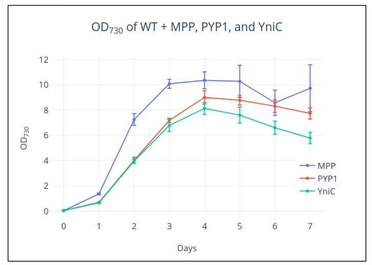
Fig 4. HPLC curve showing production of sorbitol and ribose, a side product due to phosphatase promiscuity

Methods

- Plasmids containing MPP, sorbitol-6-phosphate dehydrogenase, antibiotic resistance, and lac operon are transformed into PCC 7002.
- Cells induced with IPTG at OD_{730} = 0.05.
- Cells are grown for 7 days at 150 µE and 1% CO_2.
- 500 µL of DI water is added each day for evaporation losses.
- 400 µL of culture is sampled each day and OD_{730} is measured.
- An enzymatic sorbitol assay kit is used to find sorbitol concentration in the media.

Results

Gene	Full Name	Organism
MPP	Mannitol Phosphatase	*Eimeria tenella*
PYP1	Polyol Phosphatase 1	*Saccharomyces cerevisiae*
YniC	HAD-like Phosphatase	*Escherichia coli*



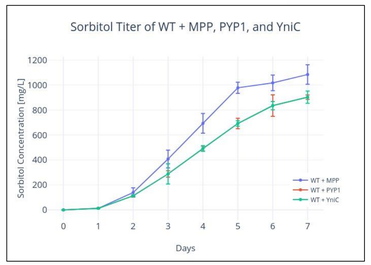
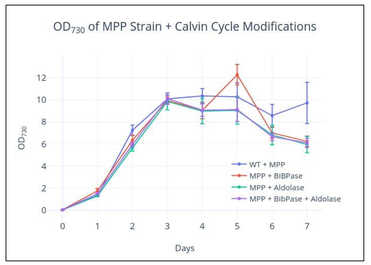


Fig 5. Growth of Wild type + various phosphatases (left) and resulting sorbitol titer (right).



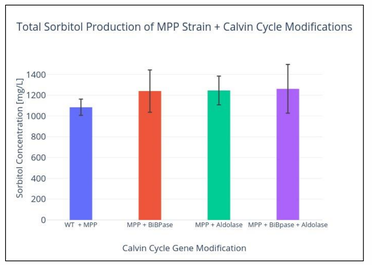


Fig 6. Growth of MPP strain + Calvin Cycle modifications (left) and resulting sorbitol titer (right).

Conclusion

- Cells that contain MPP show a better growth profile. This is because Mannitol-1-Phosphatase is a highly substrate specific enzyme that only works on polyol phosphates **(Fig 5)**.
- The final concentration of sorbitol (Day 7) of the MPP strain is higher than the PYP1 and YniC strains, further indicating MPP's substrate specificity and ability to convert Sorbitol-6-Phosphate into Sorbitol **(Fig 5)**.
- The WT + MPP strains has the best growth curve of all the Calvin Cycle modifications, indicating that the BibPase and Aldolase changes may put more strain on the overall carbon distribution to biomass from the Calvin Cycle **(Fig 6)**.
- There is no statistical significance on the total sorbitol production, when comparing the WT + MPP strains and the Calvin Cycle modification strains **(Fig 6)**.

Future Work

- Since the MPP strains is producing more sorbitol, membrane sorbitol transporters need to tested and characterized to see if sorbitol secretion and growth of the cells improve.
- Since self-shading is an issue for cyanobacteria, new methods of gene expression control for the sorbitol biosynthetic pathway need to be tested to improve long-term sorbitol production and lifespan of the cells.

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