

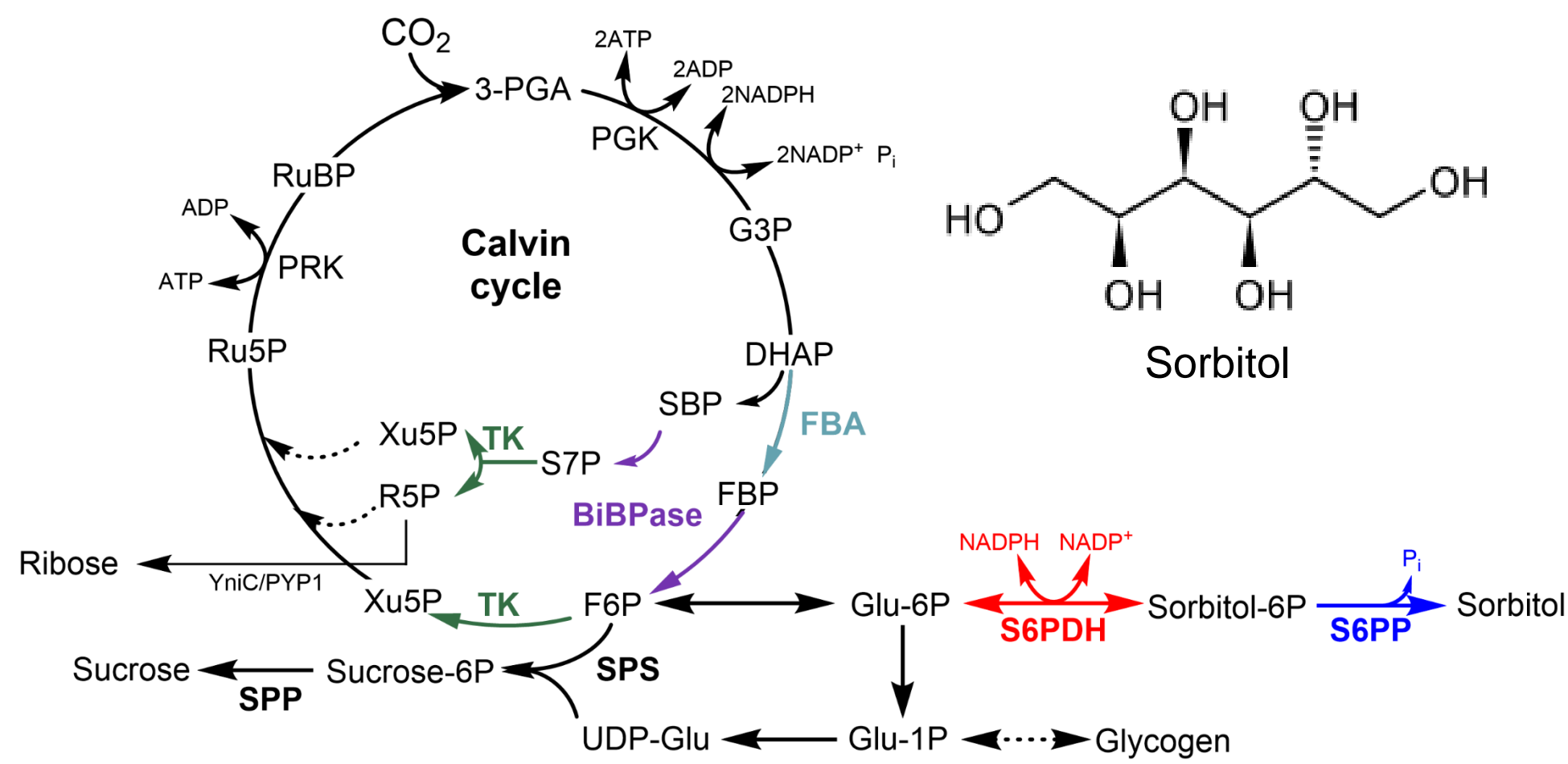


Metabolic Enhancements for the Photosynthetic Production of Sorbitol in Cyanobacteria

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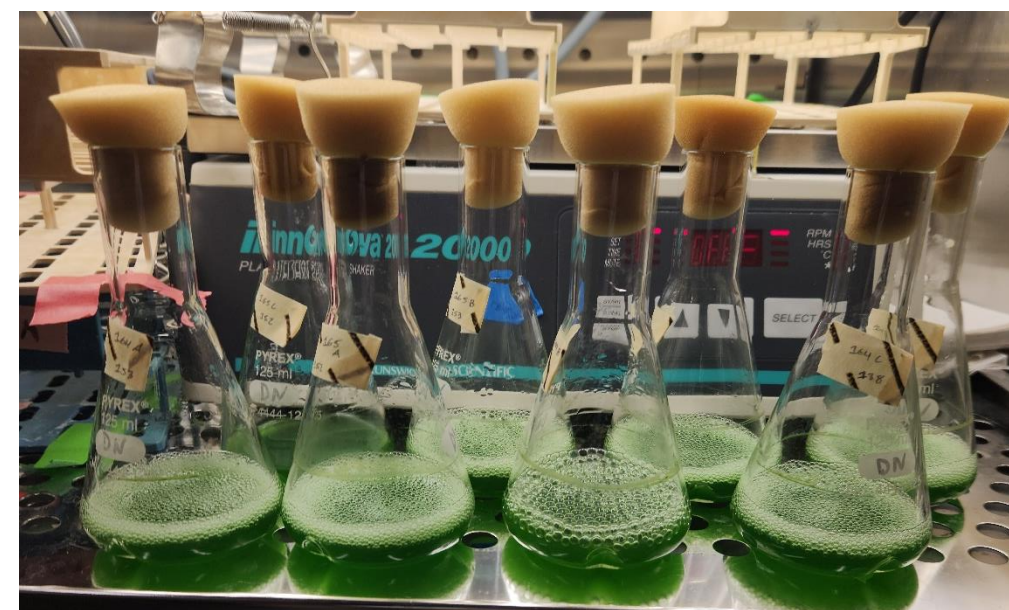
Sorbitol Biosynthetic Pathway in Cyanobacteria



- Sorbitol is a sugar alcohol commonly used as a sweetener.
- Recognized by U.S Dept. of Energy as a target chemical for bioproduction.
- Also used in the cosmetics, pharmaceuticals industries and can be polymerized for bioplastics and polymers.
- Can be produced from fructose-6-phosphate.
- 2 gene pathway
 - Sorbitol-6-phosphate dehydrogenase
 - Sorbitol-6-phosphate phosphatase
- Apple trees have a polyol phosphatase that is specific to sorbitol-6-phosphate.
 - Enzyme is yet to be sequenced.

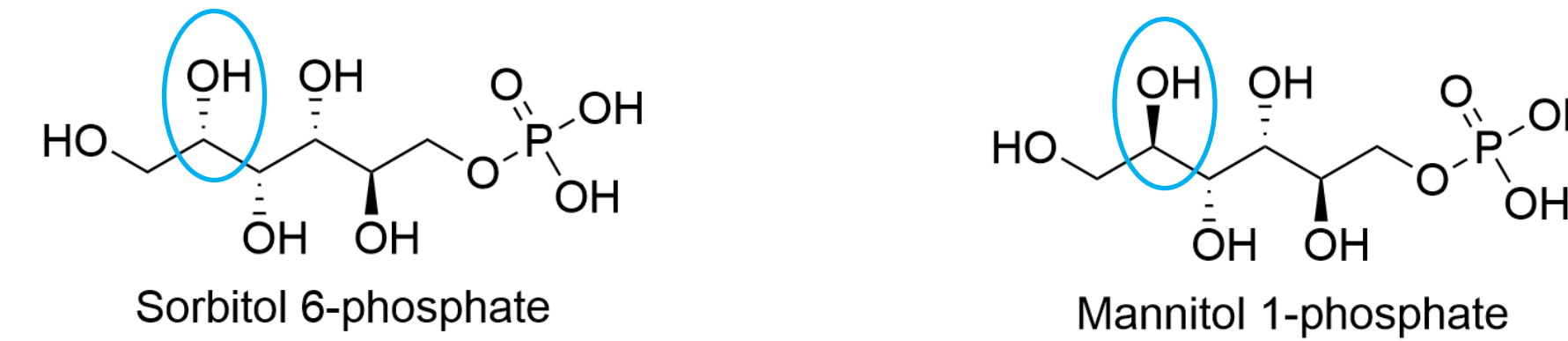
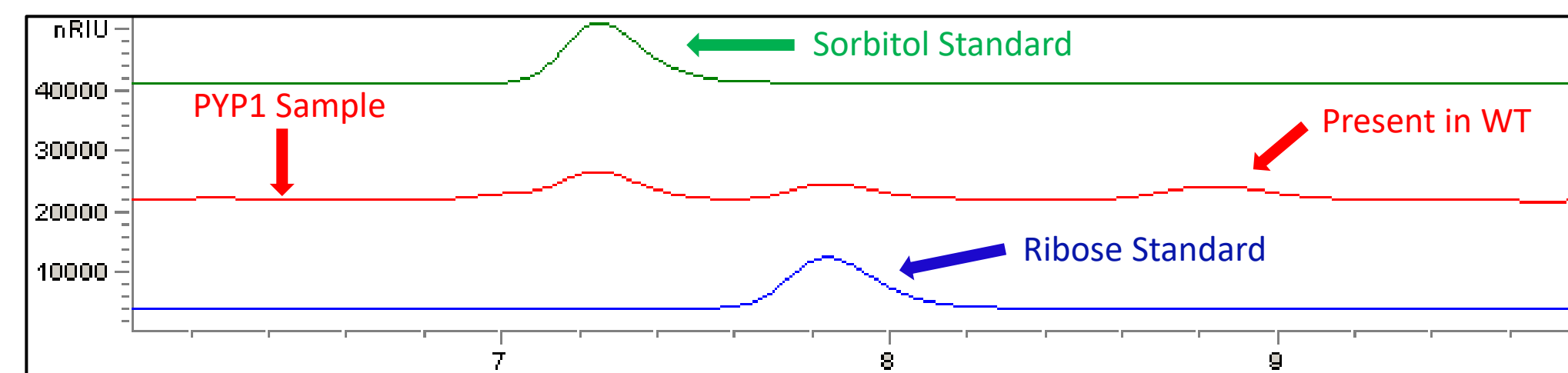
Plasmid	Relevant characteristics
pCMK8	<i>S. cerevisiae</i> PYP1 + <i>M. domesticus</i> S6PDH on plasmid pAQ1
pCMK12	<i>E. coli</i> yniC + <i>M. domesticus</i> S6PDH on plasmid pAQ1
pCMK186	<i>E. tenella</i> M1PP + <i>M. domesticus</i> S6PDH on plasmid pAQ1
pCMK171	<i>Synechocystis</i> sp. PCC 6803 BiBPase, integrates into NS2
pCMK172	PCC 6803 Fructose-bisphosphate aldolase, integrates into NS2
pCMK173	PCC 6803 BiBPase + Fructose-bisphosphate aldolase, integrates into NS2

- Synechococcus* sp. PCC 7002 is a photoautotrophic strain of cyanobacteria.
- PCC 7002 is an excellent metabolic chassis due to its fast doubling time.
- Can tolerate various light intensities, salt concentration, and temperatures, thus making it a versatile and industrializable strain of cyanobacteria.

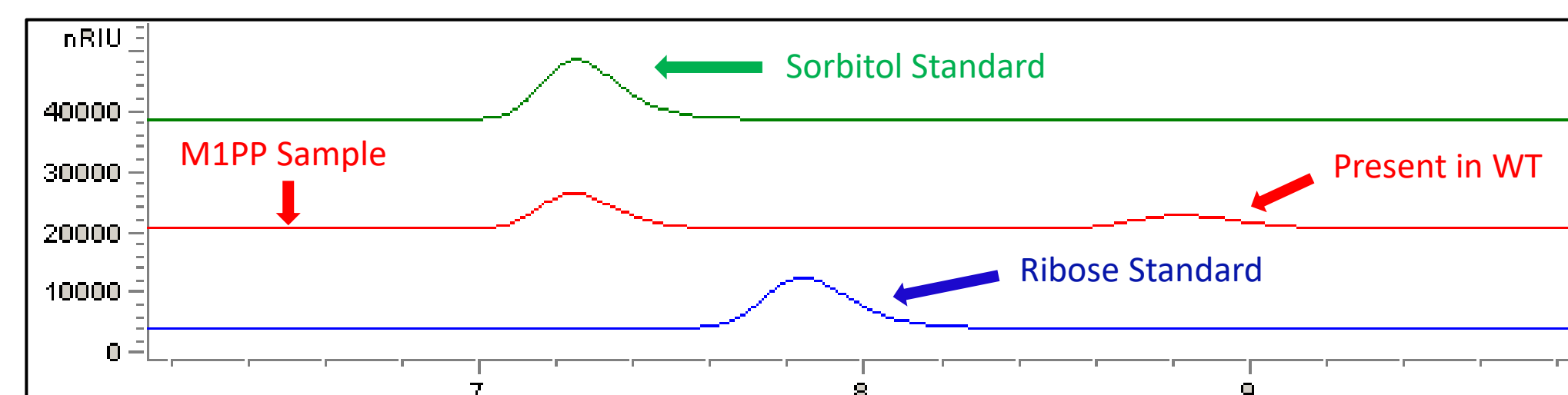
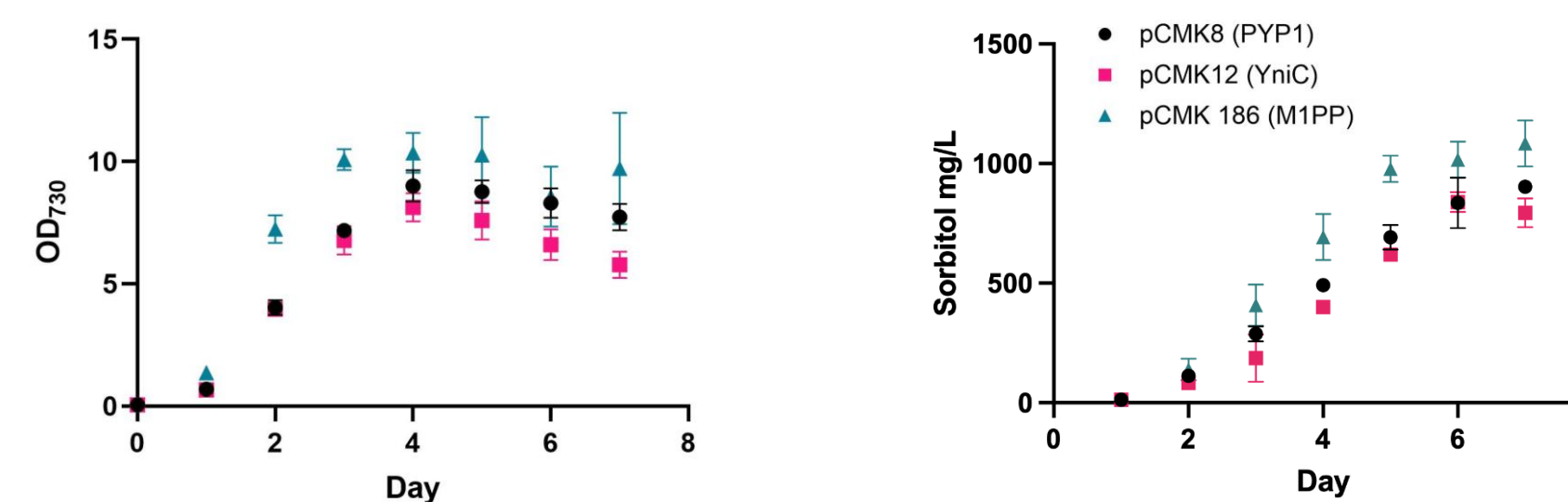


Mannitol-1-Phosphate Phosphatase is Active on S6P

- PYP1 and YniC are promiscuous phosphatases. The low substrate specificity allows for dephosphorylation of multiple sugar phosphates.
- PYP1 and YniC lead to ribose production in PCC 7002. PCC 7002 does not naturally catabolize ribose.
- Ribose synthesis lowers carbon flux through the Calvin cycle and nucleotide biosynthesis.
- Cell growth rapidly declines after 96 hours.
- HPLC shows ribose as a metabolic product.

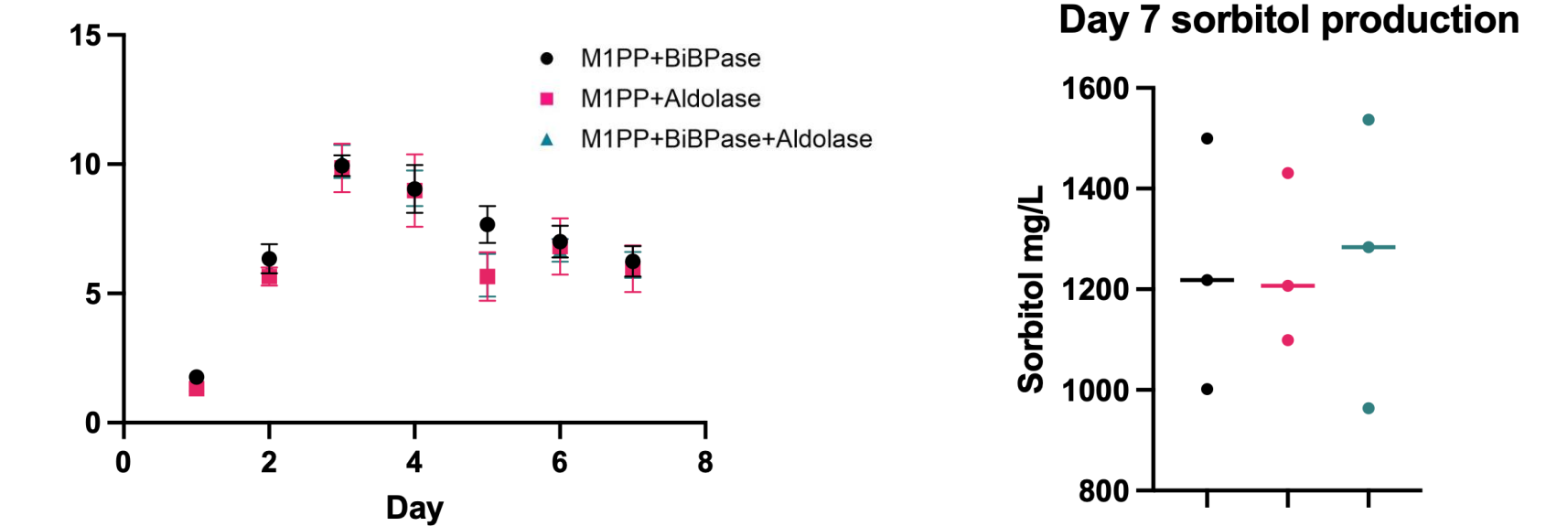


- Mannitol-1-phosphate (M1P) and sorbitol-6-phosphate (S6P) are stereoisomers.
- Mannitol-1-phosphate phosphatase (M1PP) dephosphorylates mannitol-1-phosphate to mannitol with high substrate specificity.
- Hypothesis is that M1PP can catalyze the dephosphorylation of sorbitol-6-phosphate to sorbitol since the hydroxyl configuration change is far from the target phosphate group.



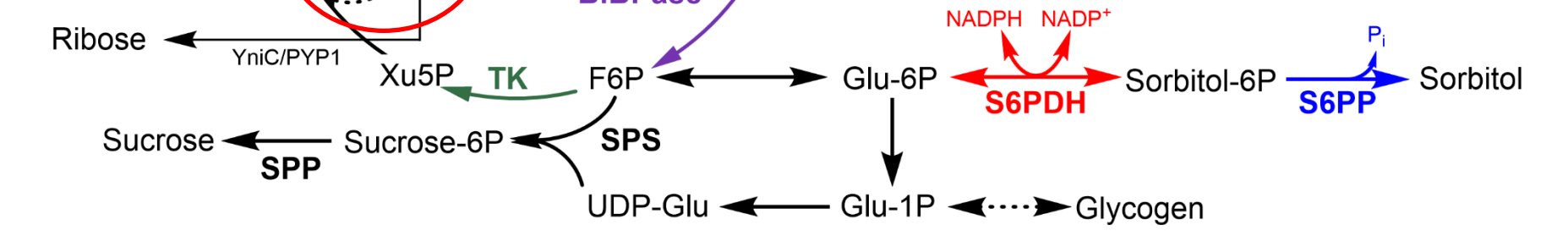
Debottlenecking the Calvin Cycle to Increase Production

- Extra copies of bifunctional fructose-1,6-bisphosphatase/sedoheptulose-1,7-bisphosphatase (BiBPase) were integrated into the chromosome.
- Fructose-bisphosphate aldolase (Aldolase) was also integrated into the chromosome.
 - Genes were integrated individually or as a BiBPase-Aldolase operon.
- Overexpression increases sorbitol titer, but not co-expression.
 - Upstream Calvin cycle bottlenecks likely exist.



Future Work

- Overexpress upstream Calvin cycle genes (*prk*, *pgk*, *rbclX*) in combination with BiBPase.
- Characterize enzyme activity of M1PP.
- Determine active site of M1PP.
- Elucidate S6PP from apple.
- Downstream optimization for maximized sorbitol production.



Acknowledgments

- Dr. Cody Kamoku and Dr. David Nielsen for their exceptional mentorship.
- Luis Taquillo for conducting experiments on media optimization.
- Grace Reed for helping with sorbitol assaying.
- Collin Travis for helping with the debottlenecking experiments and HPLC analysis.
- Fellow peers in the Varman and Wang labs.
- Fulton Undergraduate Research Initiative (FURI) for supporting undergrad research at ASU.



CBET-1705409