

# Engineering *Corynebacterium glutamicum* with Malonate Pathway for Optimizing Flavonoid Production



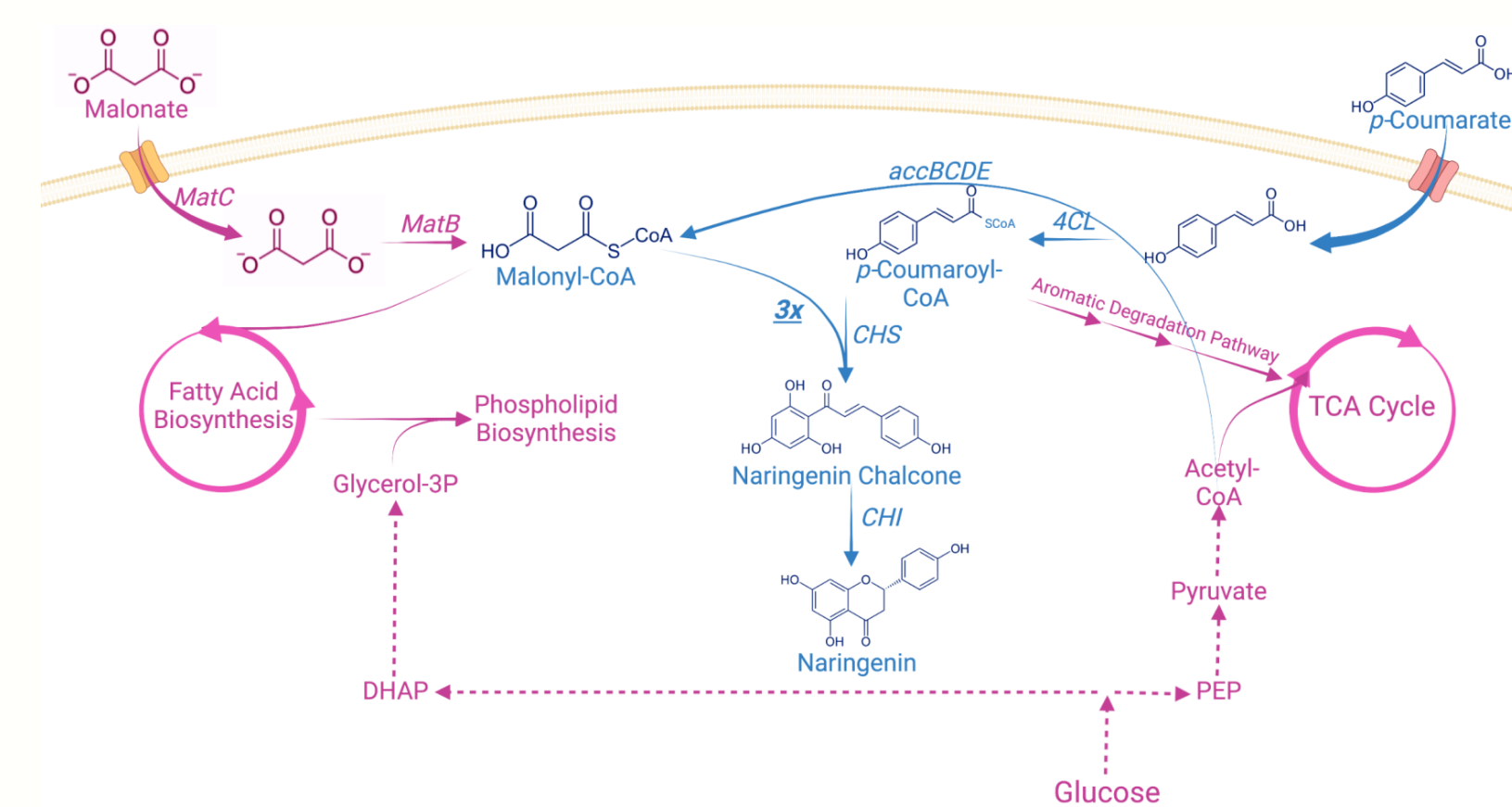
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## Motivation

Naringenin is a phytochemical flavonoid with antioxidant, antitumor, antiviral, anti-inflammatory, anti-adipogenic, and cardioprotective properties [1].

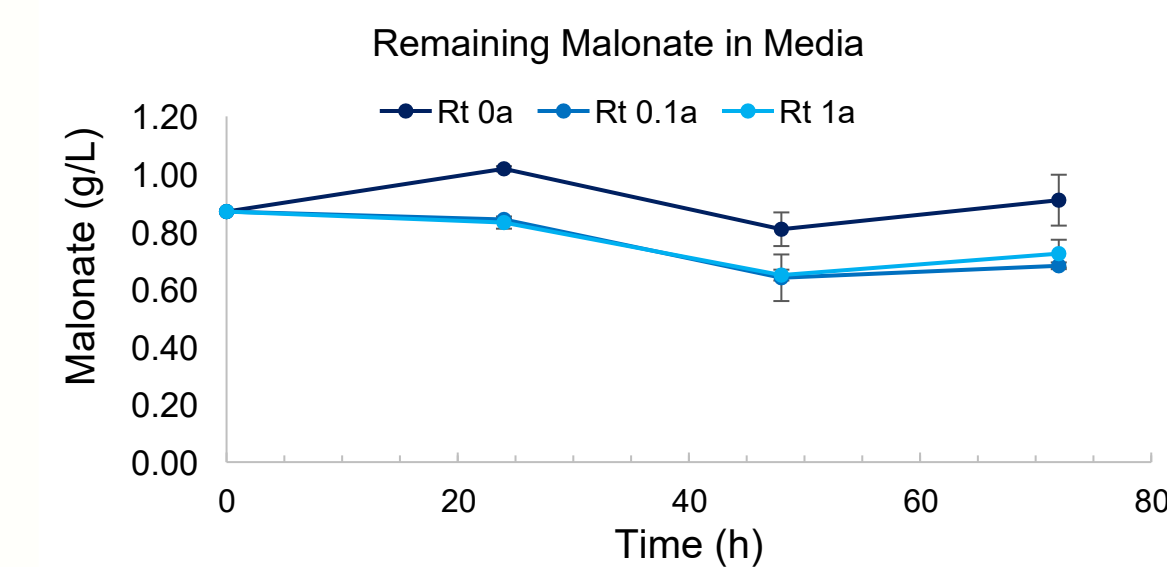


### *C. glutamicum* Pathway

- Two different malonate pathways (*R. trifoli* and *S. colicolor*) were tested to optimize expression in *C. glutamicum* strains in order to increase Malonyl-CoA supply and ultimately, naringenin output
- Features the entire deletion of the aromatic degradation pathway
- Malonyl-CoA is the bottleneck for naringenin production

## Expressing Malonate Pathway in *C. glutamicum*

- IPTG induces MatB synthetase, aTc induces MatC protein transporter
- OD<sub>600</sub> measures relative cell growth



### Malonate Consumption by Engineered Strains

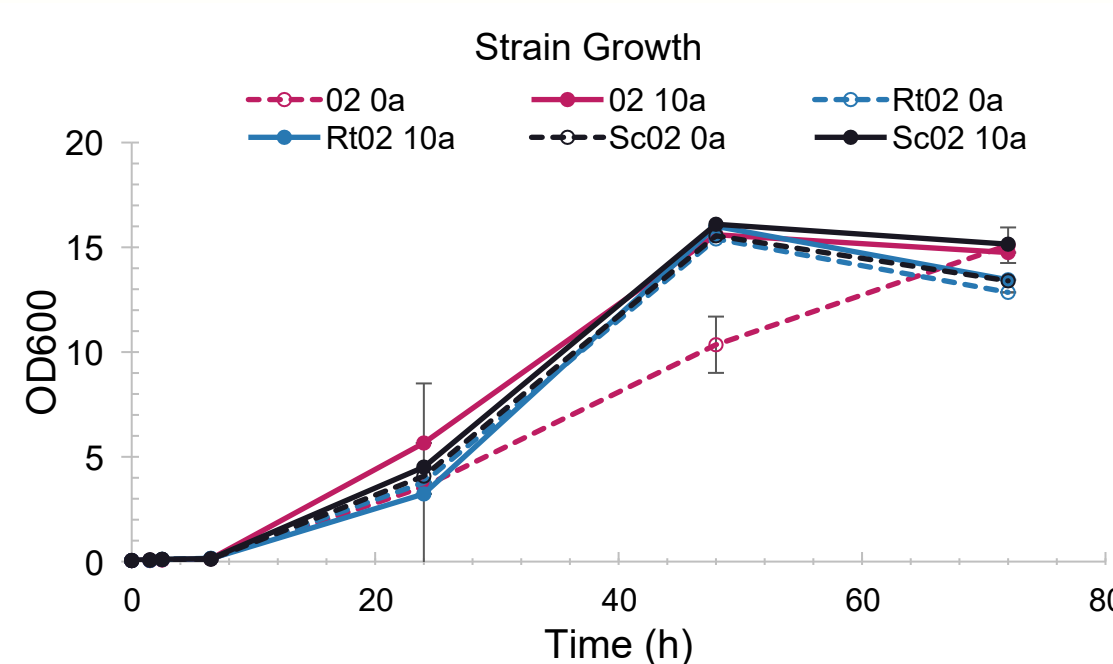
- IPTG: 0, 0.1, 1 mM
- aTc: 10 ng/mL
- Induced at OD<sub>600</sub>: 0.1

- Minimal malonate consumption
- Slightly more consumption when induced with 0.1 and 1 mM IPTG

### Growth Kinetics of Malonate Strains

- IPTG: 1mM
- aTc: 0, 10 ng/mL
- Induced at OD<sub>600</sub>: 0.25-0.3

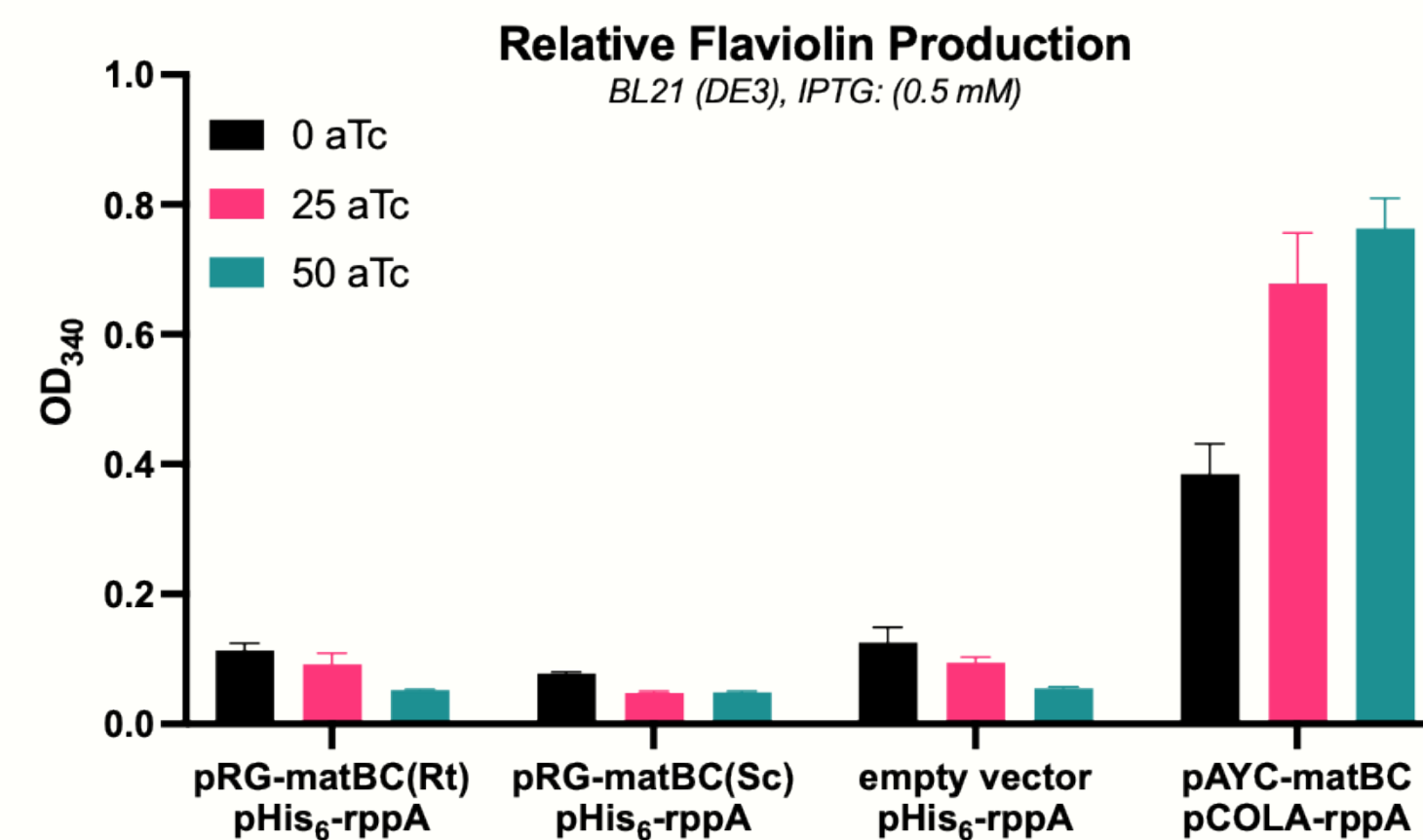
- Feeding malonate should increase cell growth
- There is no malonate consumption



### Ineffective Overexpression of Malonate Pathway

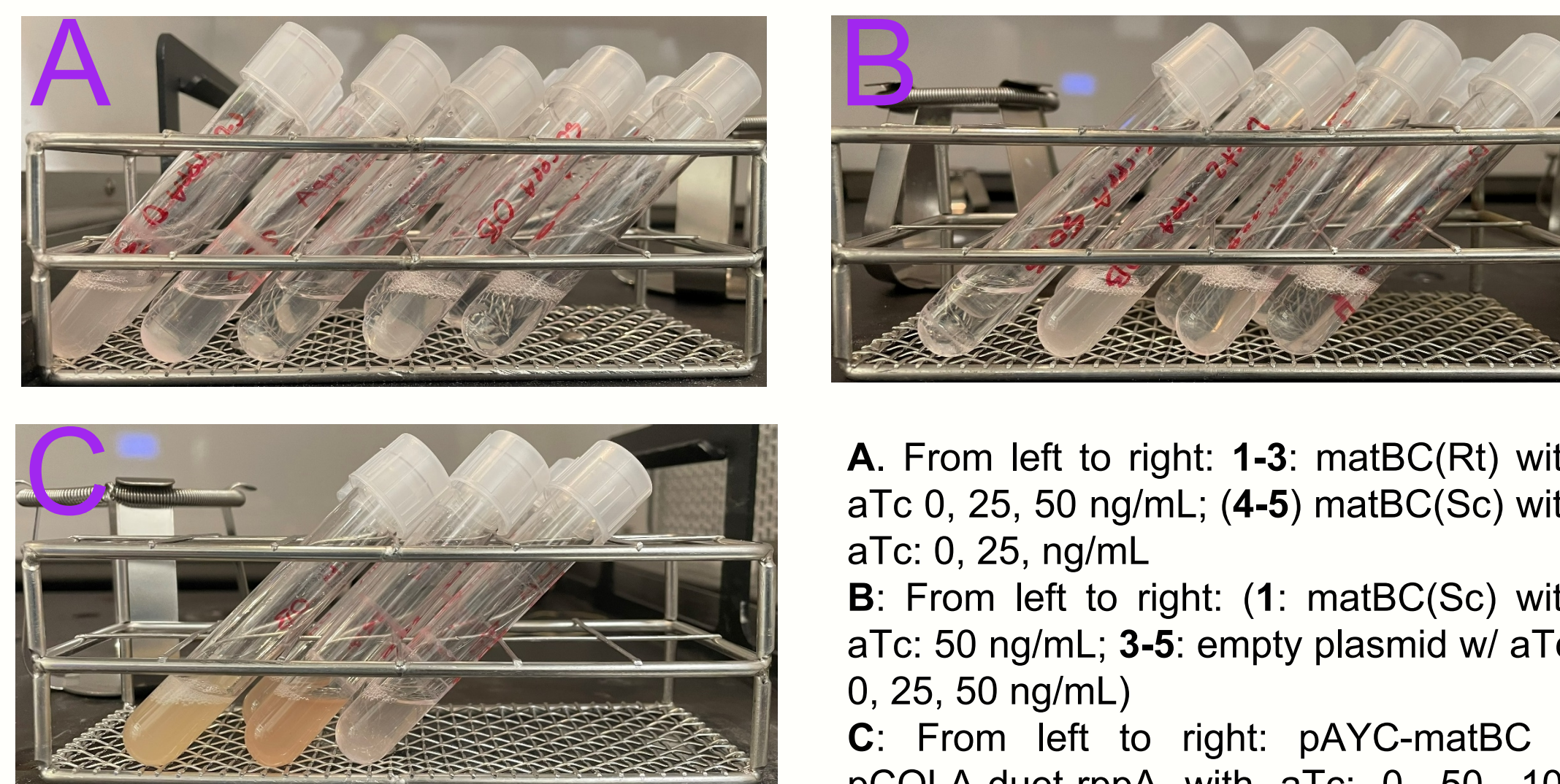
- There was minimal malonate consumption despite relative cell growth
- Glucose concentrations decreased to 0 mg/L at the 24h mark
- Glucose consumption can be attributed to cell growth, not malonate
- Once it was observed that the malonate pathway was not assimilating the malonate, this pathway was tested in *E. coli* BL21 since it is known to work in *E. coli*

## Troubleshooting Malonate Pathway in *E. coli*



### Induction of Malonate Pathway Using IPTG and aTc

- OD<sub>340</sub>: relative flavolin production
- Should see the same OD of relative flavolin as our strain control with the uninduced group



- A.** From left to right: 1-3: matBC(Rt) with aTc 0, 25, 50 ng/mL; (4-5) matBC(Sc) with aTc: 0, 25, ng/mL
- B.** From left to right: (1: matBC(Sc) with aTc: 50 ng/mL; 3-5: empty plasmid w/ aTc: 0, 25, 50 ng/mL)
- C.** From left to right: pAYC-matBC + pCOLA-duet-rppA with aTc: 0, 50, 100 ng/mL. These two plasmids are known to work in *E. coli* cells, hence the production of a pink solution.

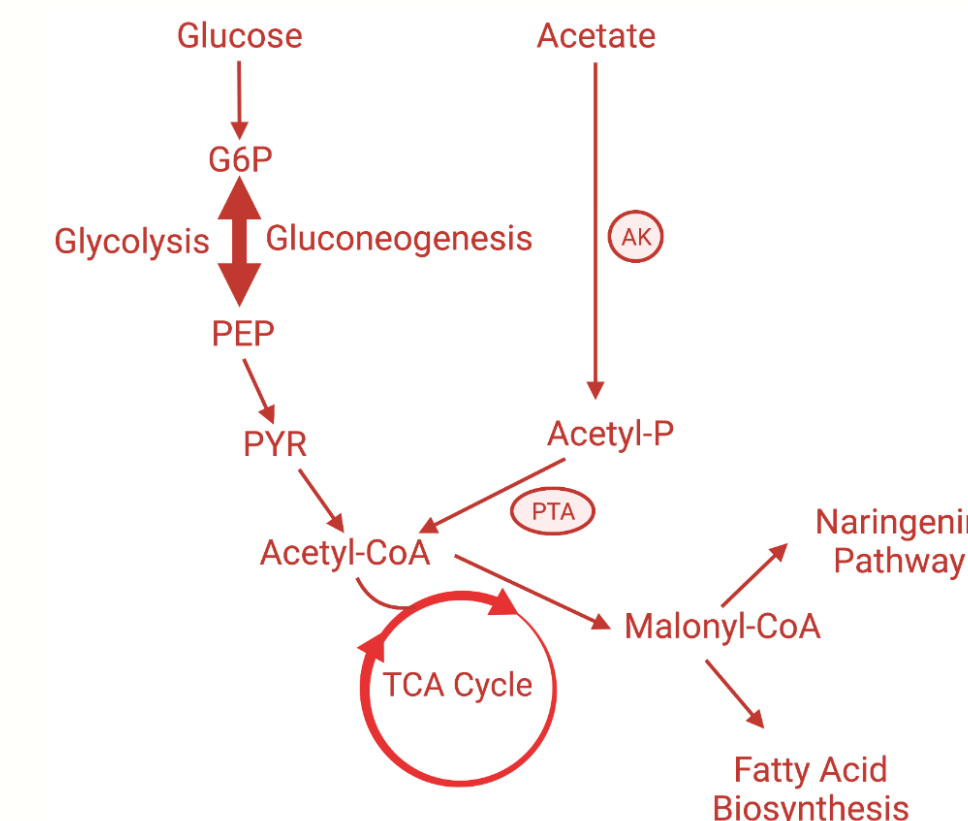
- Expected all to turn pink, indicating an active malonate pathway
- All experimental groups remained clear or cloudy white
- 0 ng/mL aTc controls should have had baseline pink color because the cells are already producing Malonyl-CoA, but the incorporation of our pathway should produce more

### Poor rppA Plasmid Expression

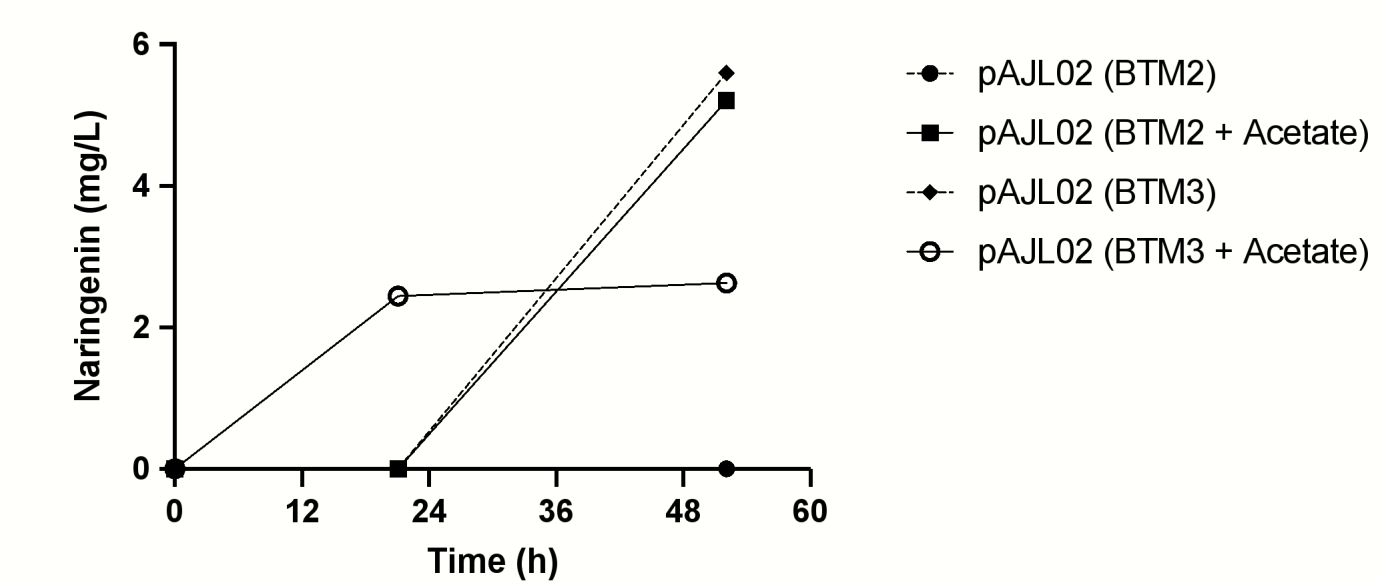
- Uninduced group is not making a baseline Malonyl-CoA, suggesting poor expression of the rppA plasmid
- Experimental solutions did not turn pink, indicative of improperly functioning rppA plasmid

## Feeding Acetate

Feeding acetate in an alternative approach to feeding malonate. Moreover, it could be fed simultaneously with malonate to further increase the Malonyl-CoA titer.



### Comparing Naringenin Production (1 g/L Acetate; BTM2 vs BTM3)



### Acetate Increases Naringenin Production

- BTM3 has additional peptone, providing an additional carbon source
- Expected to see highest naringenin yield in BTM3 with acetate
- BTM3 had higher naringenin yields than BTM2
- Highest naringenin production observed in BTM3, followed by BMT2 + Acetate

## Future Work

- Concentrations of IPTG and aTc can be adjusted to determine optimal induction levels
- Test a new rppA plasmid where we have seen colonies turn pink before, and if there is no decrease in malonate quantity over time, this will indicate that our malonate pathway is not compatible with *C. glutamicum*

## Acknowledgements

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## References

- Salehi, B., Fokou, P. V. T., Sharifi-Rad, M., Zucca, P., Pezzani, R., Martins, N., & Sharifi-Rad, J. (2019). The therapeutic potential of naringenin: a review of clinical trials. *Pharmaceuticals*, 12(1), 11.