Modeling of the engineered production of curcumin in *Escherichia coli*

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Introduction

- Curcumin is the major bioactive natural product in turmeric (*Curcuma longa*), which is commonly used as a food additive (flavor and colorant) and traditional medicine for thousands of years.
- Antioxidant[1], anti-cancer[2], anti-allergic[3], anti-inflammatory[4], and anti-Alzheimer's[5] effects
- Current production relies on extraction of producing plants: requires large quantities of farmland and organic solvents[6]
- Microbial production represents a great alternative: saves time and materials.
- Microorganisms can be engineered to produce curcumin by incorporating curcumin biosynthetic enzymes.
- Testing various parameters in lab experiments is time-consuming and labor-intensive.
- This work aims to establish a computer model to simulate the production of curcumin in *Escherichia coli.*

Enzyme Kinetics Data

Enzyme	Substrate	K_m (μM)	k_{cat} (1/s)	Source
TAL	L-tyrosine	1492.2	155	[7]
4CL	*p*-coumaric acid	26	88.68	[8]
4CL	caffeic acid	44	31.4	[8]
4CL	ferulic acid	27	126	[8]
C3H	*p*-coumaric acid	8	10.2	[9]
C3H	*p*-coumaroyl-CoA	8	10.2	[9]
COMT	caffeic acid	68.75	0.092	[10]
COMT	caffeoyl-CoA	83.04	51.22	[10]
DCS	feruloyl-CoA	46	0.02 (n=1.8)	[11]
CURS	feruloyl-CoA	18	0.018333	[11]

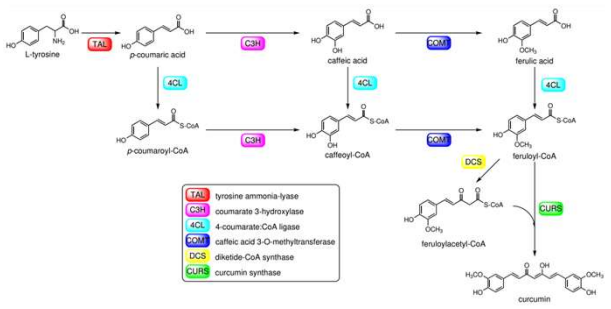

Figure 1: Full pathway from TAL to curcumin

Probabilistic Model Construction

- The PRISM Probabilistic Model Checking language
  - Continuous-time Markov chain (CTMC).
- Two assumptions made
  - The model is unaffected by the cell's central metabolism
  - Byproducts of DCS and CURS are negligible
- Each reaction is a probabilistic transition
  - The ratio of rates determines the probability a reaction will occur
  - A reaction being chosen causes an update in concentration
- Rates are calculating with the Michaelis-Menten and Hill equations

Michaelis-Menten Equation

$$rate = \frac{K_{cat} * [E] * [S]}{K_m + [S]}$$

Hill Equation

$$rate = \frac{K_{cat} * [E] * [S]^n}{K_m^n + [S]^n}$$

- Code snippet

```
module partial_pathway
[] fcoa > 0  -> v1: (facoa' = facoa + 1) & (fcoa' = fcoa – 1);
[] fcoa > 0 & facoa > 0  ->  v2:(cur' = cur + 1) & (fcoa' = fcoa – 1) &
      (facoa' = facoa – 1);
endmodule
```

Results

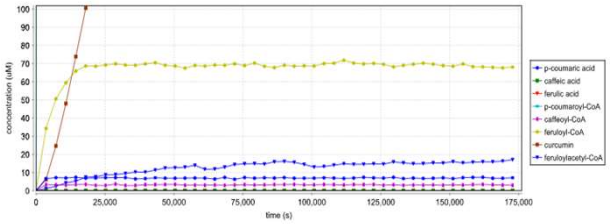

Figure 2. Concentrations of all substrates/products over a 48 hour period

- An average of 100 simulations resulted in 486.565 mg/L curcumin yield.
- C3H may be the limiting enzyme due to rate of *p*-coumaroyl-CoA production
- Overexpression modeling analysis was done to test C3H hypothesis: overexpressing C3H to 50 mg/L increased the curcumin yield 32.7%.

- All combinations of two or three enzymes changed to 10 mg/L or 50 mg/L were simulated to determine the optimum concentration of enzymes (See table below)

Enzyme_concentration (mg/L)	Curcumin yield (mg/L)
C3H_50, DCS_50, CURS_50	957.7
4CL_10, C3H_50, CURS_50	733.2
All at 25	486.6
C3H_10, DCS_50, CURS_10	140

- This data shows that C3H, DCS, and CURS are the three most limiting enzymes and increasing them will increase the final curcumin yield.
- C3H, DCS, and CURS have been marked as further optimization targets

Conclusions and Future Work

- More work is to be done to determine more accurate kinetic parameters for all reactions
- More products will be added to the model (curcuminoids)
- Using the information from our representative model, we will validate the model in the lab.

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References

1. Menon, V. P., et al. Antioxidant and anti-inflammatory properties of curcumin. 2007.
2. Vera-Ramirez, L., et al. Curcumin and liver disease. 2013.
3. Chaniyilparampu, R. N., et al. Curcuminoids and its metabolites for the application in allergic ocular/nasal conditions. 2014.
4. Lestari, M. L., et al. Chapter three – curcumin. 113-204.
5. Goozee, K. G., et al. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. 2016.
6. Pawar, H., et al. A novel and simple approach for extraction and isolation of curcuminoids from turmeric rhizomes. 2018.
7. Cui, P., et al. Characterization of two new aromatic amino acid lyases from actinomycetes for highly efficient production of *p*-coumaric acid. 2020.
8. Rao, G., Pan, X., Xu, F. et al. Divergent and overlapping function of five 4-coumarate/coenzyme a ligases from *Populus tomentosa*. 2015
9. Schoch, G., et al. CYP98A3 from Arabidopsis thaliana is a 3'-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway, 2001.
10. Ma, Q., Xu, Y. Characterization of a caffeic acid 3-O-methyltransferase from wheat and its function in lignin biosynthesis, 2008
11. Katsuyama, Y., et al. Curcuminoid biosynthesis by two type III polyketide synthases in the herb *Curcuma longa*. 2009

GitHub code