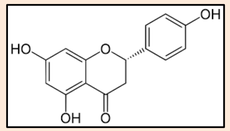
Engineering *Corynebacterium glutamicum* for the production of naringenin

Andrew Reed, Chemical Engineering
Mentor: Arul Mozhy Varman, Assistant Professor of Chemical Engineering
School for the Engineering of Matter, Transport, and Energy

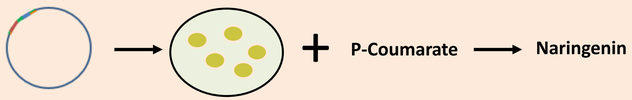
Purpose

Naringenin is an incredibly promising chemical, but difficult and inefficient to produce.

Naringenin is a flavonoid that has demonstrated anti-Alzheimer's and anti-inflammatory properties, found in grapefruit and herbs



The current methods used to isolate flavonoids is very inefficient and material intensive, inhibiting its usage
This study was conducted to create a method of naringenin production through *C. glutamicum* via constructed plasmid insertion



Due to the endogenous aromatic degradation pathway of *C. glutamicum*, it was the ideal cell type to use

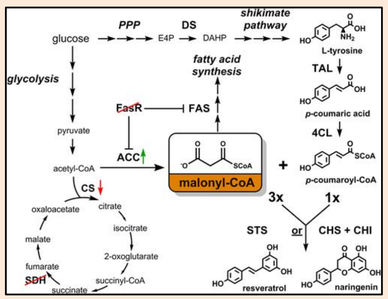
Background

Many factors could impact the performance of this study so rigorous research was required

Corynebacterium glutamicum is a gram-positive bacterial cell type that naturally creates L-glutamate and resists aromatic toxicity
The chosen feed substate for this study was p-coumarate, a naturally aromatic compound that can be consumed by *C. glumaticum* for cell growth

The genes that were used to construct the plasmids used were 4CLpc, 4CLat, CHI, CHSat, and CHSph, which when combined allows for p-coumarate to be converted into naringenin

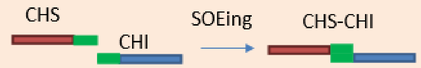
In the metabolic pathway for naringenin production there is a bottleneck which requires an abundance of malonyl-CoA in order to produce naringenin, making malonyl-CoA overproduction a necessity



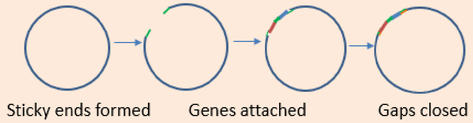
Plasmid Construction

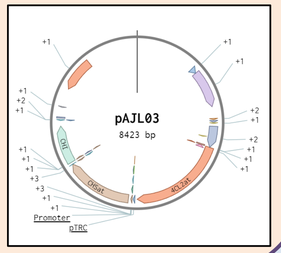
Four plasmids were devised that would allow the cells to convert p-coumarate into naringenin, the difference between them being the sources of the genes used

In order to improves the accuracy of Gibson assembly SOEing was done to combine the CHS and CHI genes



Gibson assembly was used to combine the 4Cl, CHS-CHI, and the pCRBduet2 backbone into the desired plasmids





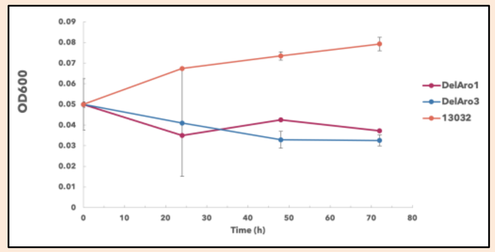
Transformation of electrocompetent cells was done through electroporation in order to incorporate the plasmids into the cells' DNA

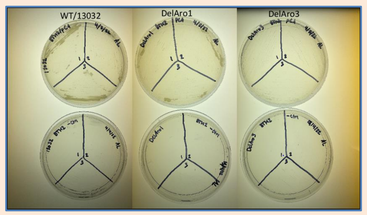
Culturing

Fermentation of the plasmid positive cells was done to confirm and characterize naringenin production

Fermentations using the strain 13032 were done with p-coumarate as the only carbon source to confirm cell growth in the presence of aromatics, which it did

Strains DelAro1 and DelAro3 were grown to see how the removal of the aromatic degradation pathways affect cell growth



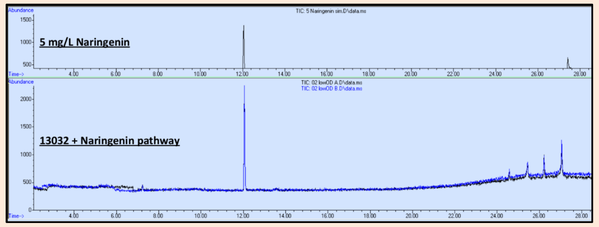


P-coumarate present growth assays were done to examine the performance of the cells and the utilization of p-coumarate

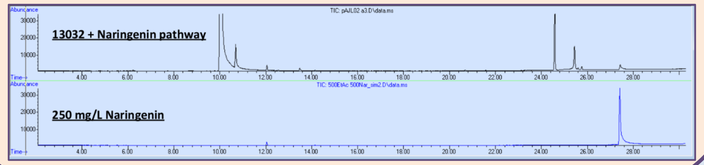
Analysis

Samples were taken from cell culture and the contents extracted from the cell mass which was characterized through GCMS and HPLC analysis

Initial GCMS analysis showed that naringenin precursors were being produce by the recombinant cells, requiring further investigation



Through rigorous investigation, it was determined that naringenin was actually being produced, but in minuscule amounts



Next Steps

What else remains to be done

Completion of pAJL04 and testing of its naringenin producing capabilities still need to be performed
Fermentations will need to be continued to optimize current naringenin production, along with those including the malonyl-CoA producing matBC pathways

Acknowledgements

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FURI

Ira A. Fulton Schools of Engineering
Arizona State University