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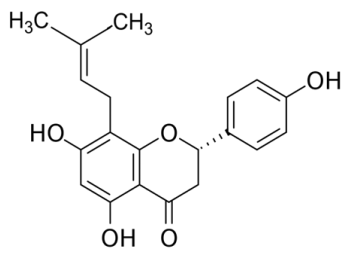
LABBELS ASSOCIATE LAB 2024 meeting

UNITING MINDS FOR FUTURE VISION

Construction of an optimized *Escherichia coli* strain able to produce prenylflavonoids

Motivation



Prenylnaringenin

Potent estrogenic activity

Other Biological activities:

- Anticancer
- Antioxidant
- Anti-viral
- Immunomodulatory
- Anti-inflammatory
- Anti-bacterial

Growing interest in its industrial production

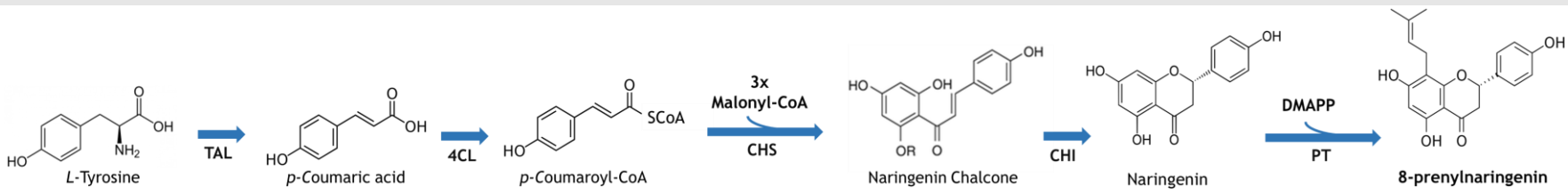
Problem: Low accumulation in plants

Find a solution!

Construction of microorganisms able to produce these compounds

Herein, we aimed to design, construct, and validate a biosynthetic pathway to produce prenylnaringenins in *Escherichia coli* for the first time

Methods & Results



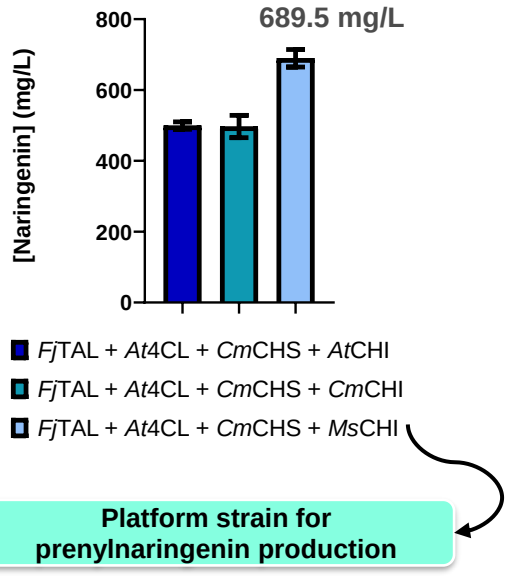
Step 1. Construction of an optimized *E. coli* strain to produce naringenin

*Fj*TAL + *At*4CL + *Cm*CHS
+
*At*CHI / *Cm*CHI / *Ms*CHI

↓

E. coli M-PAR-121 – Tyrosine-overproducing strain

Evaluate naringenin production from glucose – shake flask experiments



Platform strain for prenylnaringenin production

Step 2. Construction of DMAPP-boosted strains and validation of PT step to produce prenylnaringenin

Identification of rate limiting steps on the MEP pathway

↓ DXS and IDI genes

Integration of heterologous and native DXS and IDI genes into *E. coli* M-PAR-121 genome

↓ CRISPR-Cas12a

1. M-PAR-121:*Bs*DXS
2. M-PAR-121:*Sc*IDI
3. M-PAR-121:*Bl*IDI
4. M-PAR-121:*Bs*DXS:*Sc*IDI
5. M-PAR-121:*Bs*DXS:*Bl*IDI
6. M-PAR-121:*Ec*DXS
7. M-PAR-121:*Ec*IDI
8. M-PAR-121:*Ec*DXS:*Ec*IDI

Transformation with naringenin pathway + PT holding plasmid

Test a library of PTs:

1. *Hl*PT
2. *Sf*PT
3. CdpC3PT
4. AnaPT
5. *Cs*PT3
6. coAnaPT
7. CloQ
8. *Ec*PT
9. NphB
10. *Strep*PT
11. UbiA

99 combinations of strains/PTs

Evaluate prenylnaringenin production from glucose - 1 mL scale experiments in deep well blocks

Results:

E. coli M-PAR-121 (WT) → ✖ No prenylnaringinenin detected

DMAPP-boosted M-PAR-121 strains → ✓ Prenylnaringinenin detected in 12 different combinations of strains/PTs

Strain	PT	Prenylnaringenin
M-PAR-121:*Bs*DXS	CdpC3PT	21.6 µM
	coAnaPT	19.6 µM
	CloQ	3.3 µM
	NphB	24.7 µM
	StrepPT	37.0 µM
	UbiA	20.6 µM
M-PAR-121: *Ec*DXS	CoAnaPT	15.3 µM
	CloQ	20.6 µM
	NphB	27.5 µM
	StrepPT	19.6 µM
M-PAR-121:*Bs*DXS:*Sc*IDI	CloQ	6.2 µM
M-PAR-121:*Ec*DXS:*Ec*IDI	EcPT	49.6 µM

Conclusions

1. Construction of an optimized *E. coli* strain to produce naringenin → Highest *de novo* production reported so far in *E. coli*

2. Construction of DMAPP-boosted strains and validation of PT step to produce prenylnaringenin → DMAPP modifications in the M-PAR-121 resulted in prenylnaringenin production
Next step: Validation at flask scale

Significant step towards *de novo* production of prenylflavonoids in *E. coli*

Acronyms: 4CL: 4-coumarate-CoA ligase; AnaPT: C3-prenyltransferase from *Neosartorya fischeri*; *At*: *Arabidopsis thaliana*; CdpC3PT: 7-*O*-prenyltransferase from *Neosartorya fischeri*; CHS: chalcone synthase; CHI: chalcone isomerase; CloQ: prenyltransferase from *Streptomyces roseochromogenes*; *Cm*: *Curcubita maxima*; co: codon optimized; *Cs*: *Candida sativa*; DXS: deoxyxylulose 5-phosphate synthase; *Ec*: *Escherichia coli*; *Fj*: *Flavobacterium johnsoniae; Hl: Humulus lupulus*; IDI: isopentenyl diphosphate isomerase; MEP: methyl erythritol phosphate; *Ms: Medicago sativa;* NphB: hydroxynaphthalene Ptase; PT: prenyltransferase; Strep: *Streptomyces*; TAL: tyrosine ammonia lyase; UbiA: 4-hydroxybenzoate octaprenyltransferase.

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