# Polyketide Synthases in *Piper auritum*, *Piper nigrum*, and *Piper sarmentosum*

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## Background

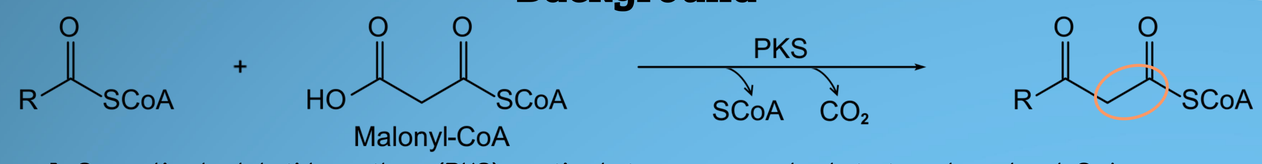


**Figure 1.** Generalized polyketide synthase (PKS) reaction between a general substrate and a malonyl-CoA unit depicting the characteristic two-carbon extension per unit on the product.

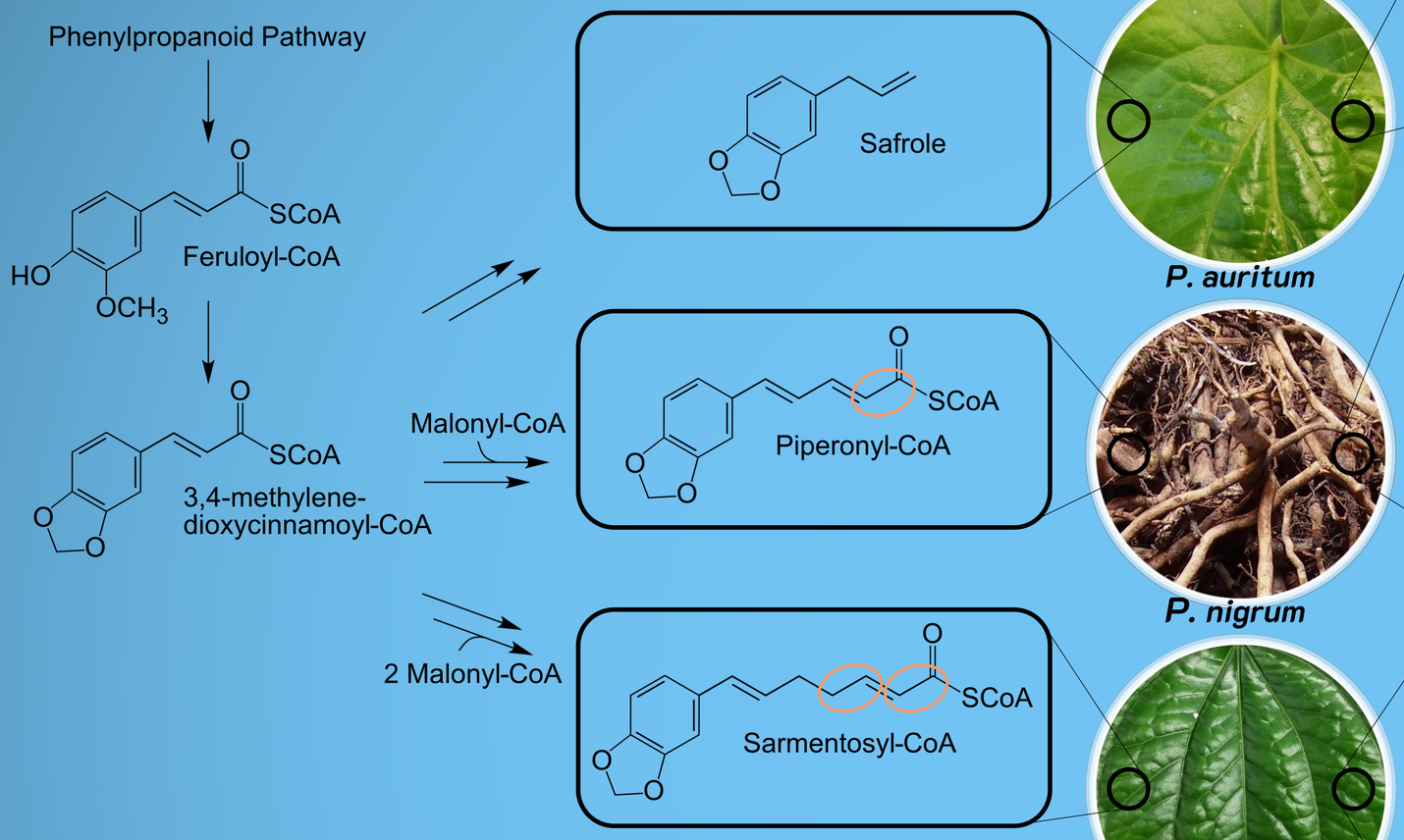


**Figure 2.** Hypothesized pathway relating safrole, piperonyl-CoA, and sarmentosyl-CoA PKS activities in *P. auritum*, *P. nigrum*, and *P. sarmentosum* respectively. Only piperonyl-CoA and sarmentosyl-CoA are polyketides and exhibit two-carbon extension(s) characteristic of PKSs.

**Table 1.** *PKS* candidates identified in *P. auritum*, *P. nigrum*, and *P. sarmentosum* transcriptomes.

| | | |
|---|---|---|
| *Piper auritum* | PaPKS1 | ——— |
| *Piper nigrum* | PnPKS1 | PnPKS2 |
| *Piper sarmentosum* | PsPKS1 | PsPKS2 |

PnPKS2 and PsPKS2 are expected to catalyze the carbon extensions since both their sequences are less homologous to chalcone synthase (CHS, Type III PKS) while not being present in *P. auritum*. All *PKS1* candidates have sequences more homologous to CHS and thus, likely function in enzyme activity that is ubiquitous in all higher plants.

## Objectives

- Amplify and sequence *PKS* candidates to verify the transcriptome assembly sequences.
- Clone *PKS* candidates into *E. coli* expression vectors for heterologous expression of the enzymes and testing of their biochemical function. PnPKS2 and PsPKS2 are hypothesized to catalyze the single and double two-carbon extension(s) to produce piperonyl-CoA and sarmentosyl-CoA respectively, from 3,4-methylene-dioxycinnamoyl-CoA.

## Methods

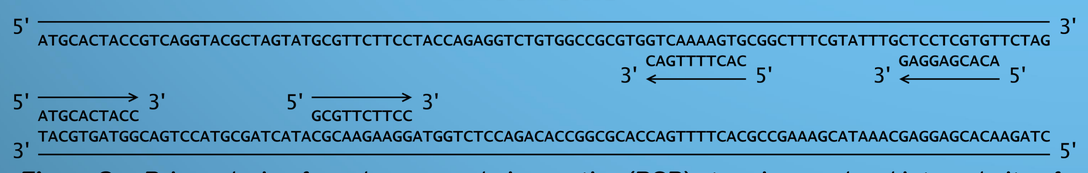


**Figure 3a.** Primer design for polymerase chain reaction (PCR) at various end and internal sites for increased chances of successful amplification.

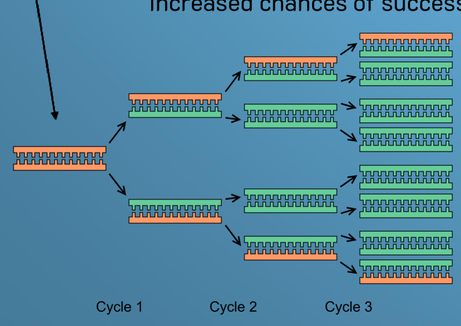


**Figure 3b.** PCR for exponential amplification of target sequence flanked by primers.

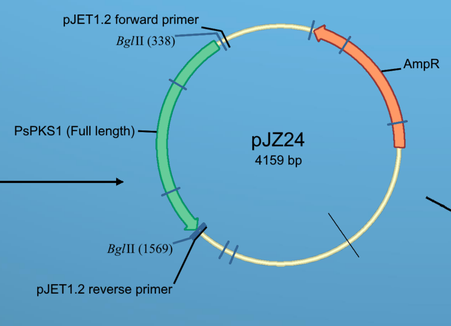


**Figure 3c.** Ligation of amplified PCR product into pJET1.2 plasmid vector.

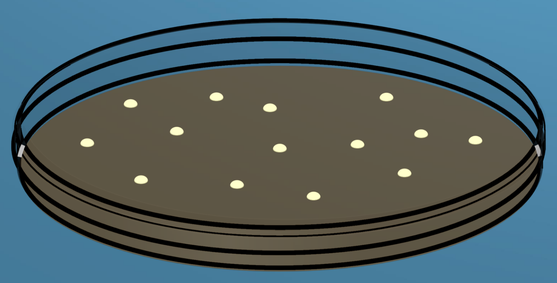

**Figure 3e.** Growth of transformed *E. coli* cells into colonies on agar plate with ampicillin resistance.

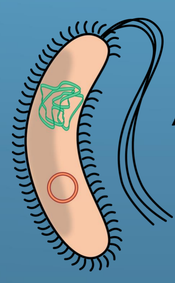

**Figure 3d.** Transformation of ligated plasmid vector into competent *E. coli* cell.

**Figure 3f.** Inoculation of one colony in LB media with ampicillin resistance.

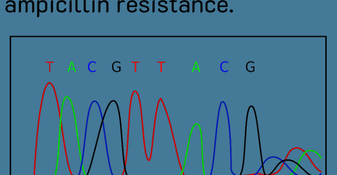


**Figure 3g.** Sequencing of miniprepped plasmids from culture.


**Figure 3h.** Sequences aligned against computer-generated assemblies for commonalities.

## Current Results

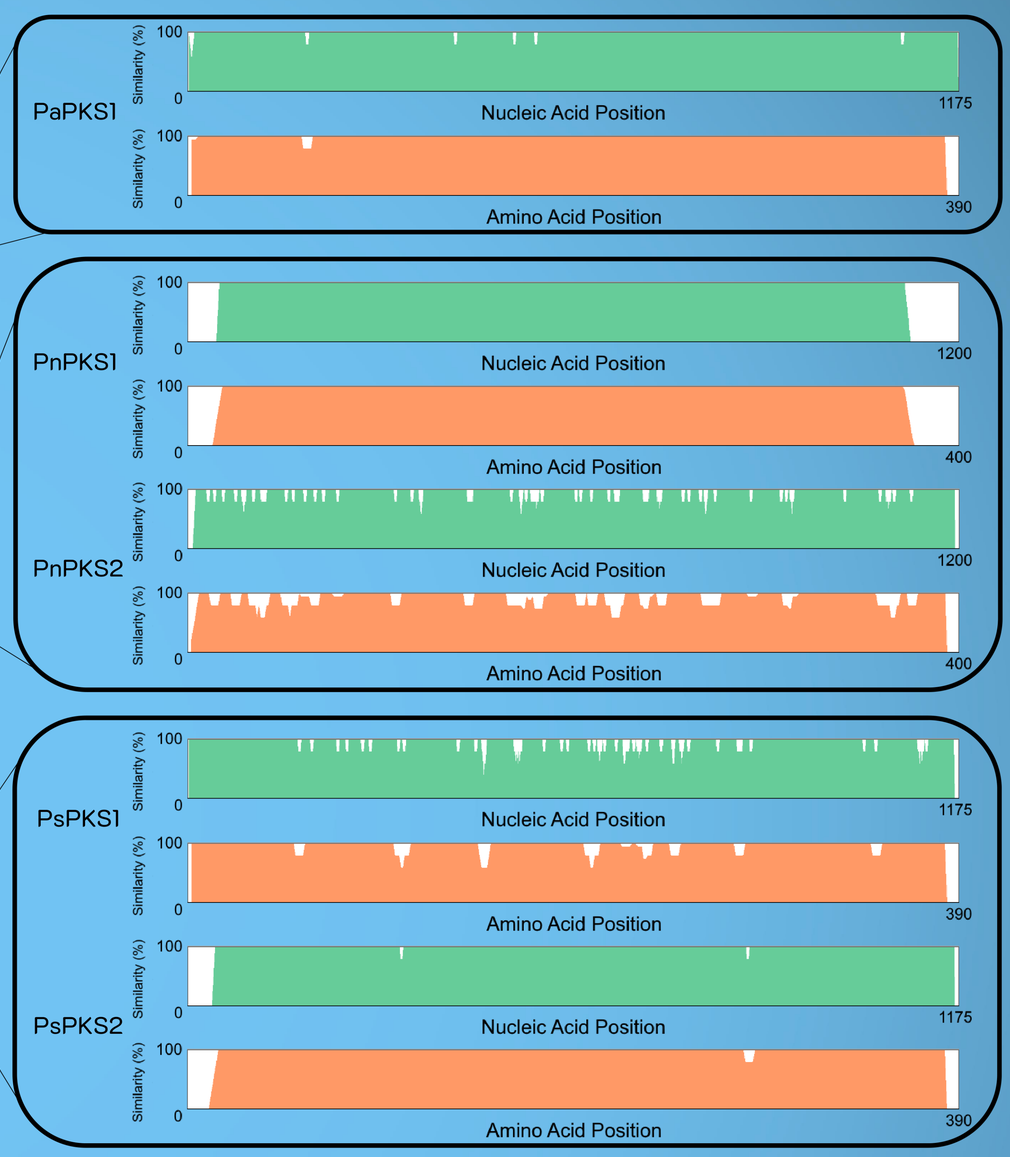


**Figure 4.** Percent similarities of transcripts (green) and translated amino acid sequences (peach) comparing *de novo* assembled transcripts with the actual sequences verified by Sanger sequencing using the amplified genes as templates. White space at the beginning and end of figures correspond to sections of *PKS* candidates yet to be sequenced.

**Table 2.** Percent similarities (green) and identities (peach) between verified *PKS*, *P. methisticum* chalcone synthase (PmCHS), and *P. methisticum* styrylpyrone synthase (PmSPS) amino acid sequences.

| Gene | PaPKS1 | PnPKS1 | PnPKS2 | PsPKS1 | PsPKS2 | PmCHS | PmSPS |
|---|---|---|---|---|---|---|---|
| PaPKS1 | | 61.3 | 76.0 | 95.2 | 82.4 | 94.2 | 81.9 |
| PnPKS1 | 72.4 | | 53.7 | 60.1 | 58.7 | 60.3 | 58.7 |
| PnPKS2 | 82.3 | 63.4 | | 76.0 | 89.8 | 74.5 | 88.3 |
| PsPKS1 | 96.7 | 71.6 | 82.0 | | 82.9 | 93.2 | 81.9 |
| PsPKS2 | 88.2 | 68.7 | 91.3 | 88.4 | | 81.4 | 93.2 |
| PmCHS | 96.2 | 72.6 | 80.3 | 95.2 | 86.2 | | 80.7 |
| PmSPS | 87.9 | 68.2 | 90.8 | 87.7 | 95.7 | 86.2 | |

As predicted, all verified *PKS1* candidates exhibit a greater homology to *PmCHS* and all verified *PKS2* candidates exhibit a greater homology to *PmSPS* than their alternate *PKS* candidate counterpart.

## Conclusions

- All *PKS* candidates were successfully confirmed with minimal differences in translated amino acid sequences relative to computer-generated assemblies.
- The transcriptome assembles produced by Matthew J.D. Doering were highly accurate.

## Upcoming Work

- Codon optimization of confirmed *PKS* candidates for expression in *E. coli*.
- Protein expression of codon-optimized *PKS* candidates in *E. coli*.
- *In vitro* confirmation of intermediary and final compound production as hypothesized.

## Future Implications

- Enhanced understanding of the function of PKSs in plant systems.
- Optimization for scale-up production of safrole, piperonyl-CoA, and sarmentosyl-CoA.
- Increased marketability of intermediary and final compounds as proposed in pathway.

## Acknowledgement

- Matthew J. D. Doering (M.Sc.)

## Funding

University of Manitoba
faculty of SCIENCE
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invent the future
NSERC CRSNG