# Unscrambling the Biosynthetic Pathway of Pradimicin A

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## Introduction

- Pradimicin A is a novel natural product synthesized by *Actinomadura hibisca* P157-2.
- Pradimicin A is a broad spectrum fungicide and effective HIV entry inhibitor. Its unique lectin-like mechanism of action might be potential to meet the challenge of multidrug resistance.
- Previously we have identified the minimal set of enzymes (PdmABCDHKL) required for the formation of the pentangular structure of pradimicin A.

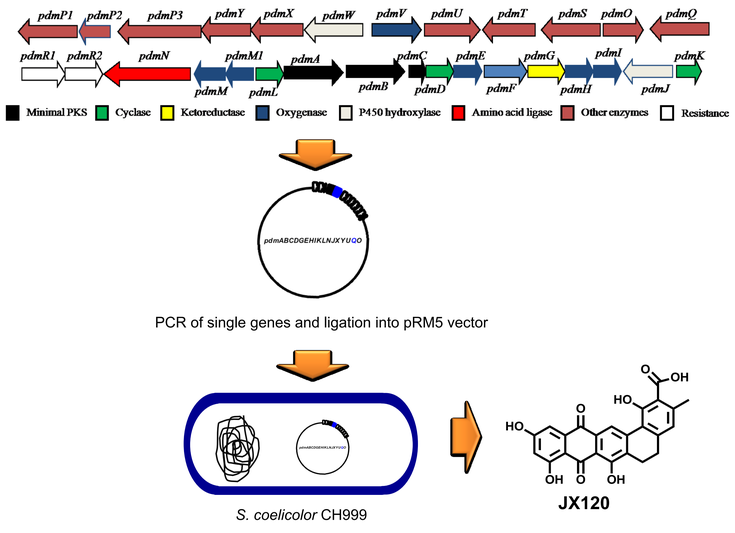
## Objectives

- To investigate the sequence of the early tailoring reactions.
- To identify the glycosyltransferases in pradimicin biosynthesis.

## Method

### *Combinatorial biosynthesis*

- PCR amplification of selected genes from the biosynthetic gen cluster of pradimicin.
- Ligation of the PCR products to the cloning vector pJET1.2.
- Ligation of the biosynthetic genes into the pRM5 shuttle vector.
- Expression of the constructs in *Streptomyces coelicolor* CH999.
- Extraction and LC-MS analysis of the products.

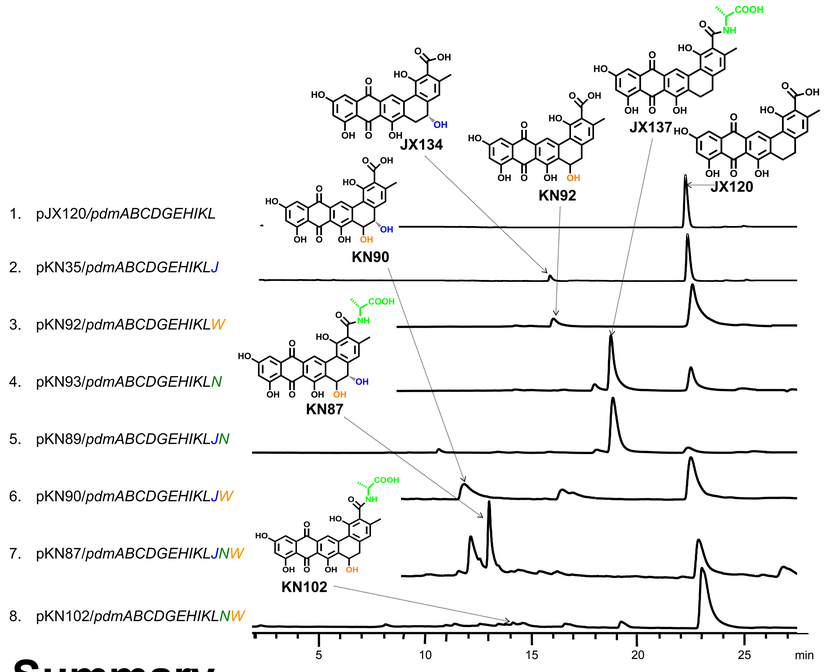



### *Gene knockout*

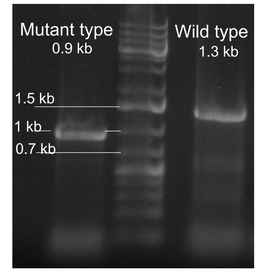
- The gene *pdmS* was disrupted via homologous recombination.
- The knockout plasmid was transferred from the donor *E. coli* ET12567 to the recipient *A. hibisca* through conjugation.

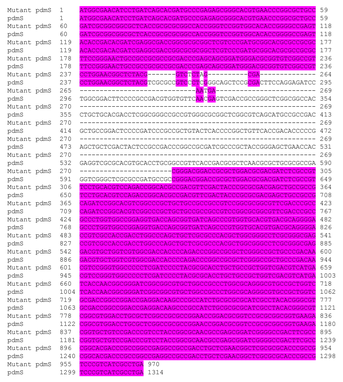
## Results

- Construction of 7 plasmids that carry a combination of the genes *pdmJ, pdmW,* and *pdmN,* together with the genes in charge of the synthesis of JX120.
- 4 new intermediates from the metabolic pathway of pradimicin were identified for the first time, KN87, KN90, KN102, and KN82.
- Functional characterization of the gene *pdmW* as a P450 hydroxylase.

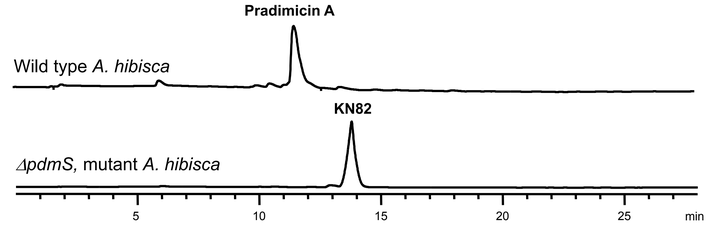



- PCR amplification of the mutant gene *pdmS* from *A. hibisca*/pKN82 and *pdmS* from wild type *A. hibisca*. The electrophoresis picture and the DNA sequence alignment of mutant *pdmS* and *pdmS* show a deletion of 0.4 kb in *pdmS.*

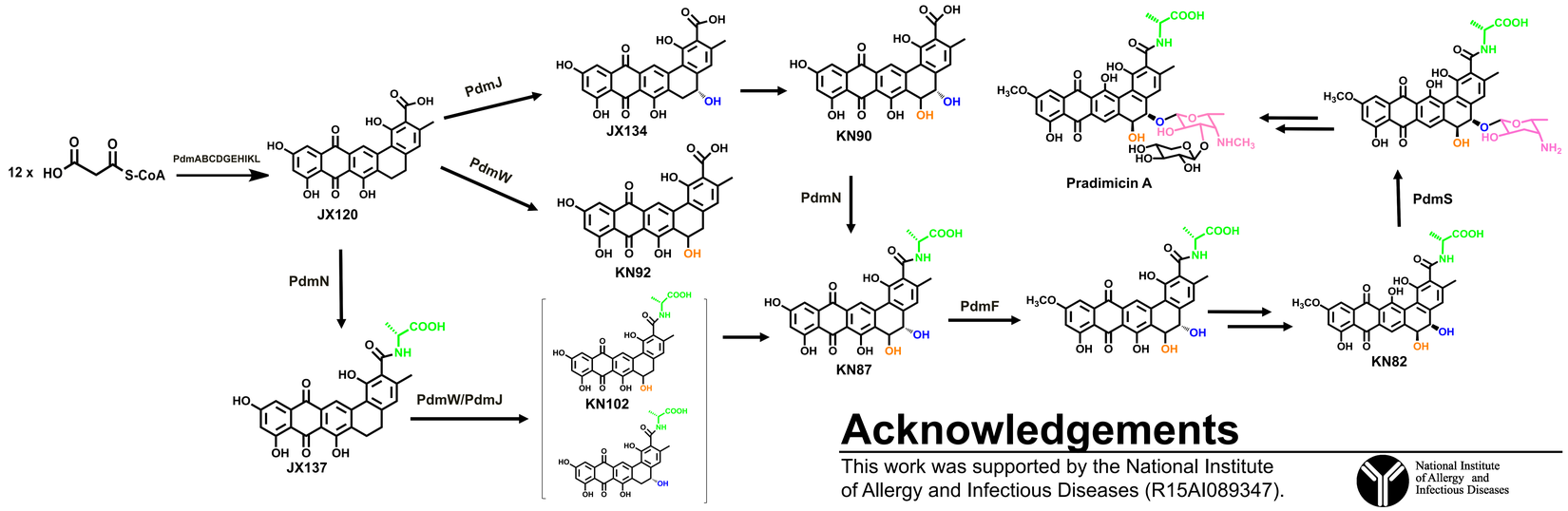


- LC/MS analysis of the compound produced by mutant type *A. hibisca*/pKN82.
- The function of *pdmS* was confirmed by gene disruption. The enzyme PdmS is the O-glycosyltransferase that attaches the first sugar moiety to the pradimicin aglycone.




## Summary




## Acknowledgements

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