

# Unscrambling the Biosynthetic Pathway of Pradimicin A

Kandy Napan<sup>1</sup>, Whitney Morgan<sup>1</sup>, Thomas Anderson<sup>2</sup>, Jon Takemoto<sup>2</sup>, Jixun Zhan<sup>1</sup>

<sup>1</sup> Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322

<sup>2</sup> Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322

## 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.

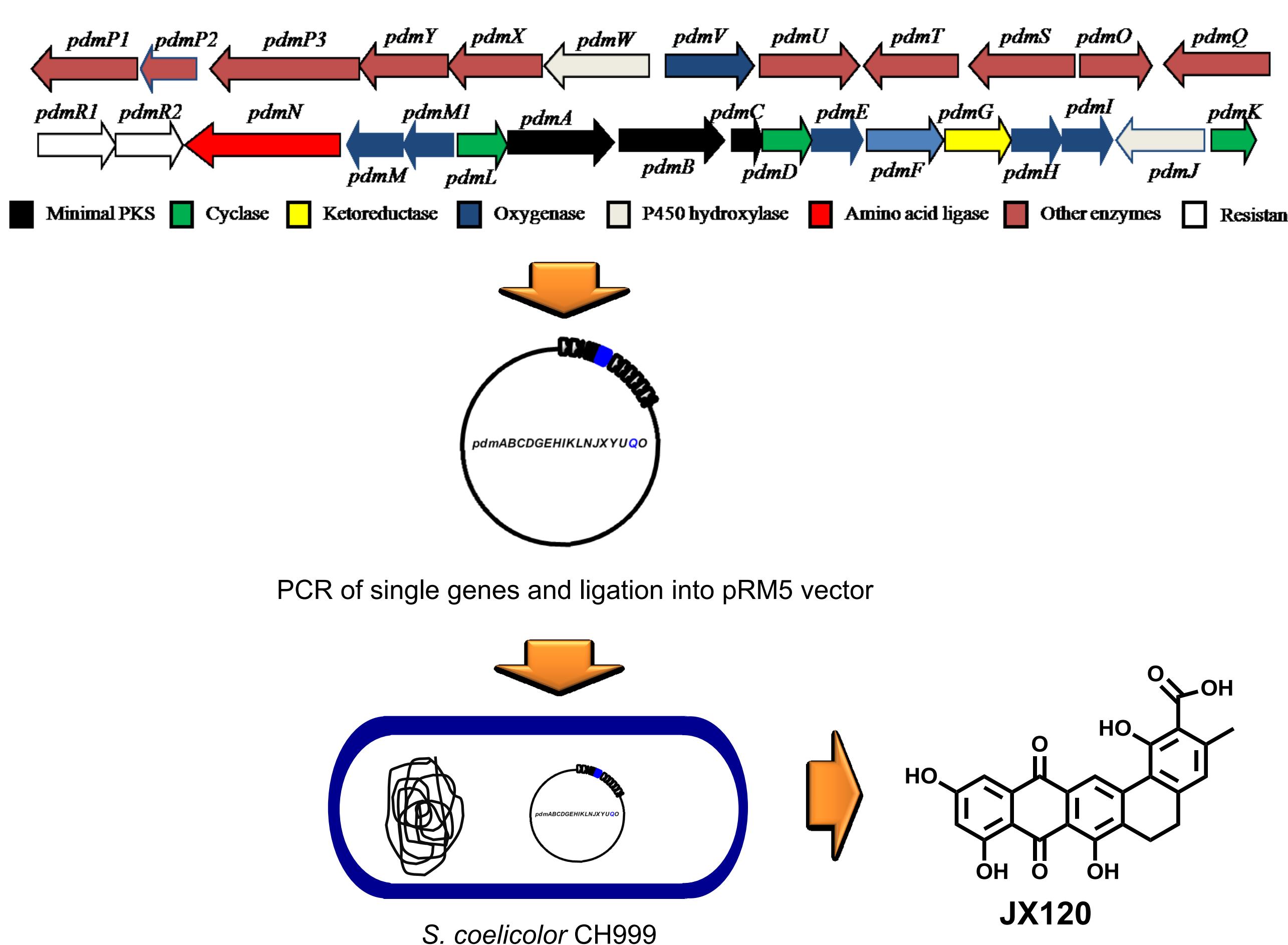
## Objectives

- 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 gene 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.

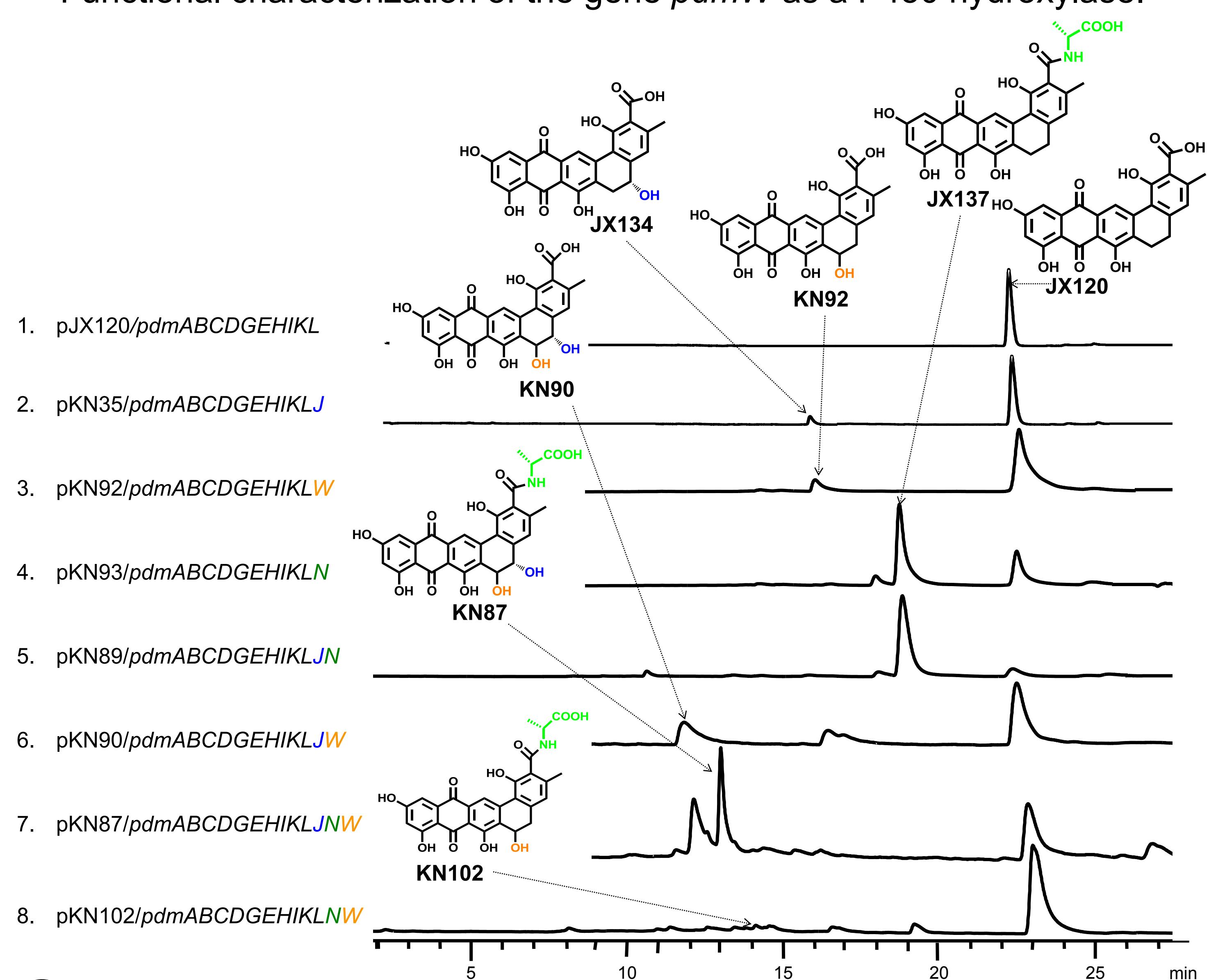


### Gene knockout

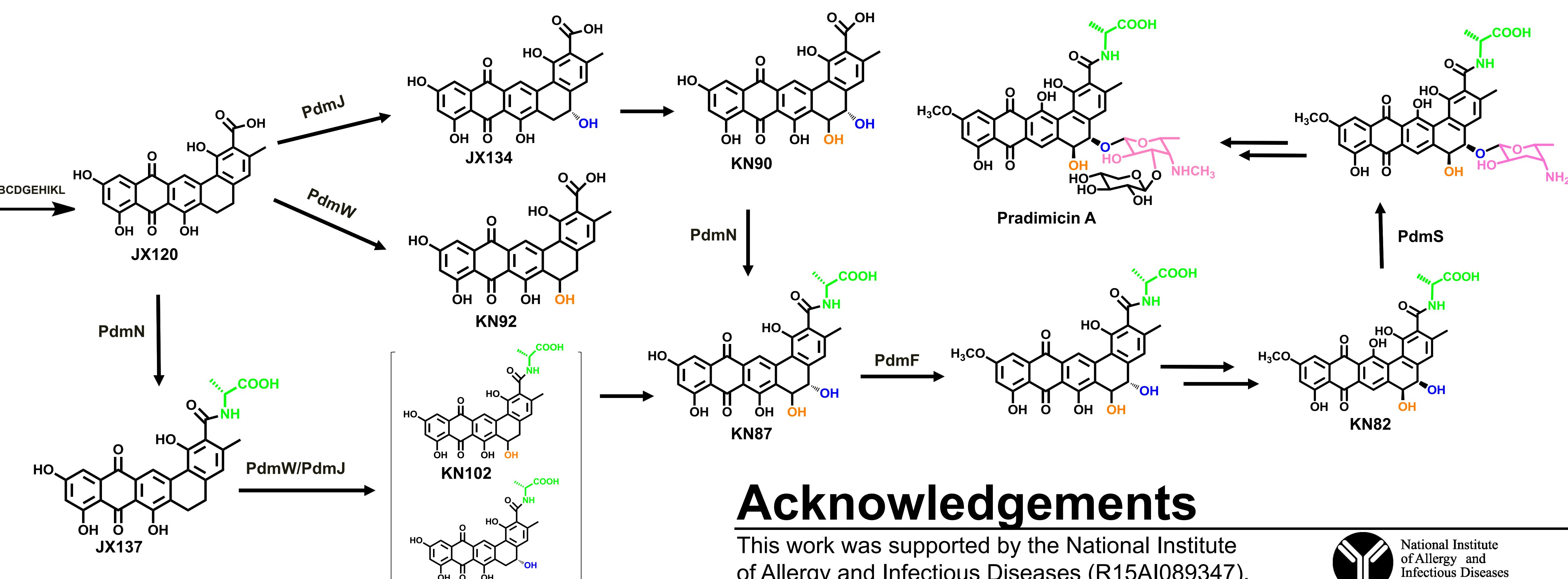
- The gene *pdmS* was disrupted via homologous recombination.
- The knockout plasmid was transferred from the donor *E. coli* ET12567 to the recipient *A. hibisca* through conjugation.

## Results

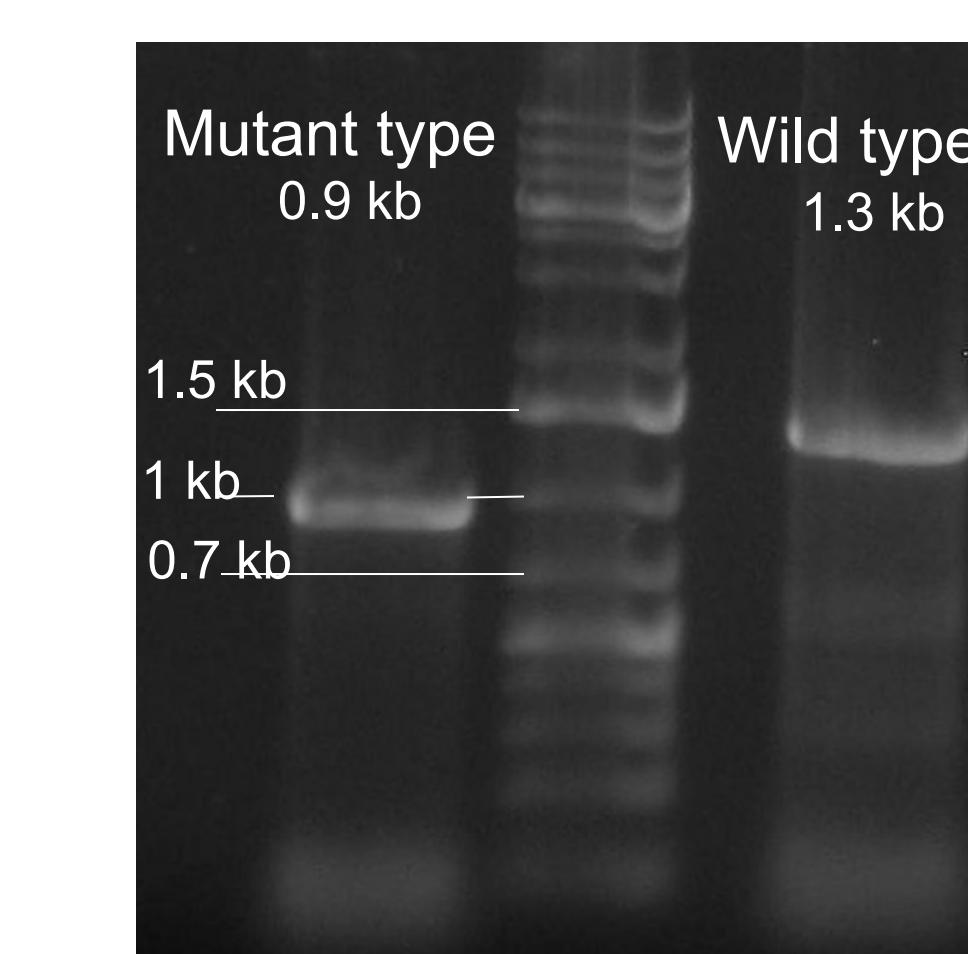
- Construction of 7 plasmids that carry a combination of the genes *pdmJ*, *pdmW*, and *pdmN*, together with the genes in charge of the synthesis of JX120.
- 4 new intermediates from the metabolic pathway of pradimicin were identified for the first time, KN87, KN90, KN102, and KN82.
- Functional characterization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



## Summary



- 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*.



	Mutant pdmS 1	Mutant pdmS 2	Mutant pdmS 3	Mutant pdmS 4	Mutant pdmS 5	Mutant pdmS 6	Mutant pdmS 7	Mutant pdmS 8	Mutant pdmS 9	Mutant pdmS 10	Mutant pdmS 11	Mutant pdmS 12	Mutant pdmS 13	Mutant pdmS 14	Mutant pdmS 15	Mutant pdmS 16	Mutant pdmS 17	Mutant pdmS 18	Mutant pdmS 19	Mutant pdmS 20	Mutant pdmS 21	Mutant pdmS 22	Mutant pdmS 23	Mutant pdmS 24	Mutant pdmS 25	Mutant pdmS 26	Mutant pdmS 27	Mutant pdmS 28	Mutant pdmS 29	Mutant pdmS 30	Mutant pdmS 31	Mutant pdmS 32	Mutant pdmS 33	Mutant pdmS 34	Mutant pdmS 35	Mutant pdmS 36	Mutant pdmS 37	Mutant pdmS 38	Mutant pdmS 39	Mutant pdmS 40	Mutant pdmS 41	Mutant pdmS 42	Mutant pdmS 43	Mutant pdmS 44	Mutant pdmS 45	Mutant pdmS 46	Mutant pdmS 47	Mutant pdmS 48	Mutant pdmS 49	Mutant pdmS 50	Mutant pdmS 51	Mutant pdmS 52	Mutant pdmS 53	Mutant pdmS 54	Mutant pdmS 55	Mutant pdmS 56	Mutant pdmS 57	Mutant pdmS 58	Mutant pdmS 59	Mutant pdmS 60	Mutant pdmS 61	Mutant pdmS 62	Mutant pdmS 63	Mutant pdmS 64	Mutant pdmS 65	Mutant pdmS 66	Mutant pdmS 67	Mutant pdmS 68	Mutant pdmS 69	Mutant pdmS 70	Mutant pdmS 71	Mutant pdmS 72	Mutant pdmS 73	Mutant pdmS 74	Mutant pdmS 75	Mutant pdmS 76	Mutant pdmS 77	Mutant pdmS 78	Mutant pdmS 79	Mutant pdmS 80	Mutant pdmS 81	Mutant pdmS 82	Mutant pdmS 83	Mutant pdmS 84	Mutant pdmS 85	Mutant pdmS 86	Mutant pdmS 87	Mutant pdmS 88	Mutant pdmS 89	Mutant pdmS 90	Mutant pdmS 91	Mutant pdmS 92	Mutant pdmS 93	Mutant pdmS 94	Mutant pdmS 95	Mutant pdmS 96	Mutant pdmS 97	Mutant pdmS 98	Mutant pdmS 99	Mutant pdmS 100	Mutant pdmS 101	Mutant pdmS 102	Mutant pdmS 103	Mutant pdmS 104	Mutant pdmS 105	Mutant pdmS 106	Mutant pdmS 107	Mutant pdmS 108	Mutant pdmS 109	Mutant pdmS 110	Mutant pdmS 111	Mutant pdmS 112	Mutant pdmS 113	Mutant pdmS 114	Mutant pdmS 115	Mutant pdmS 116	Mutant pdmS 117	Mutant pdmS 118	Mutant pdmS 119	Mutant pdmS 120	Mutant pdmS 121	Mutant pdmS 122	Mutant pdmS 123	Mutant pdmS 124	Mutant pdmS 125	Mutant pdmS 126	Mutant pdmS 127	Mutant pdmS 128	Mutant pdmS 129	Mutant pdmS 130	Mutant pdmS 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