Role of MAT domains in polyketide production in *E.coli*

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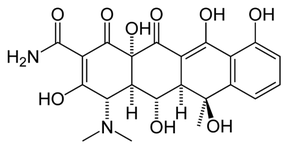
Introduction

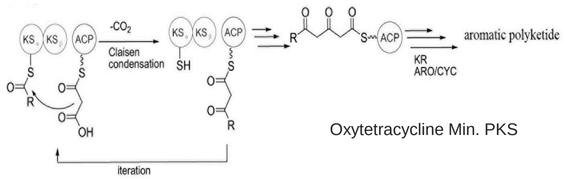
• Aromatic polyketides are a class of secondary metabolites produced by bacteria and fungi that are important antibiotic and anticancer agents. Of particular interest are the Type II polyketides including oxytetracycline and R1128.



Oxytetracycline

• Heterologous expression enables production of type II polyketide biosynthetic enzymes and their products in *E.coli.*

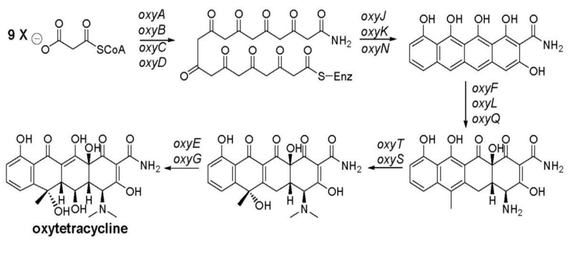
•Combinatorial biosynthesis with type II minimal PKSs has been used to make new polyketide analogs

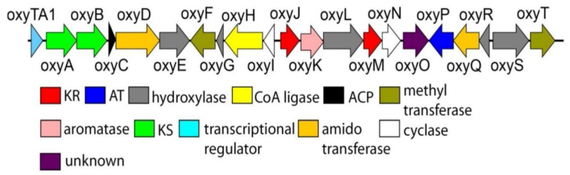


Oxytetracycline Min. PKS

• minimal PKS (polyketide synthase) generates the polyketide backbone. It includes: KS (ketosynthase), CLF (chain length factor) and ACP (acyl carrier protein)

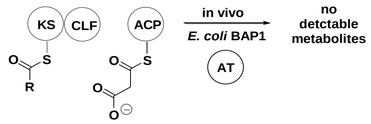
• Oxytetracycline is made from extender units of malonyl-CoA. Minimal PKS: oxyA, B, C and D





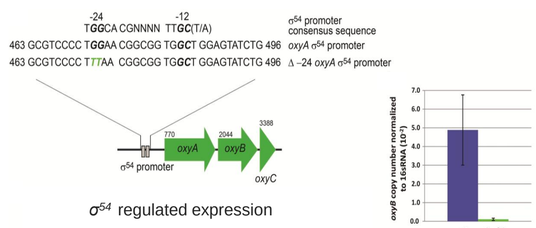
Goal: use oxytetracycline minimal PKS in E. coli to make polyketide analogs

No products from minimal PKS in *E.coli*



E.Coli BAP1 + OxyABCD

• The oxytetracycline minimal PKS native to *S.rimosus* does not produce polyketides in *E.coli*



σ^{54} regulated expression

• Minimal PKS is transcribed in *E.coli* when over expressed with σ^{54} transcription factor

• Successful heterologous expression of oxytetracycline confirms that minimal PKS proteins are functional in *E.coli*

Problem: why doesn't the minimal PKS produce polyketide products?

Hypothesis: *E.coli*'s fatty acid MAT domain cannot load oxyD, the minimal PKS ACP

Objectives

•Construct a bicistronic vector with the oxyH and oxyP MAT genes from the oxytetracycline gene cluster

•Induce polyketide production in transformed *E.coli* strains, extract metabolites analyze by LC-MS

•Determine if the putative malonyl-CoA acyltransferases (MAT), oxyH and oxyP are able to produce polyketide analogs with the minimal PKS in *E.coli*

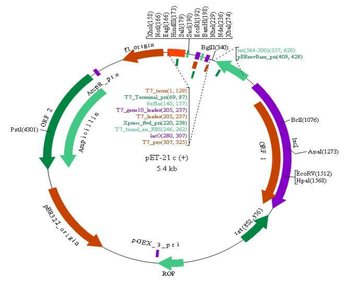
Strains to evaluate role of MAT

• *oxyH* and *oxyP* were ligated into pET21-based expression vector using restriction digests with EcoRI and XbaI to make pDCS67 and pDCS69 respectively

• Vectors were transformed into *E.coli* BAP1 along with the oxytetracycline minimal PKS (pDK04, *oxyABCD* under T7 control)

•Three bacterial strains were created:

1) Neg. control: no MAT(pET-21c + pDK04)
2) *oxyH* test (pDCS67, pDK04)
3) *oxyP* test (pDCS69, pDK04)

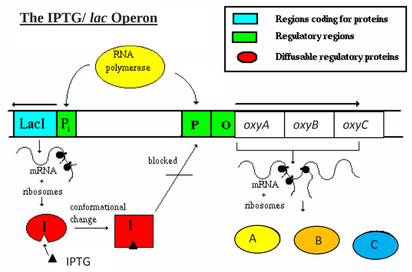


Oxytetracycline gene cluster ligated into pET-21c expression vector

Expression of MAT and minimal PKS

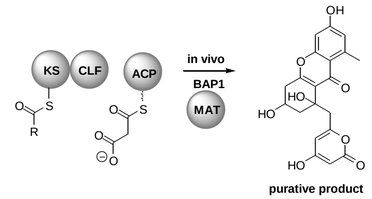
•Expression of MATs and *oxyABCD* was induced with **Isopropyl β-D-1-thiogalactopyranoside (IPTG)**

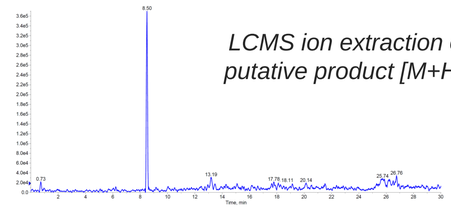
• This induces the IPTG/*lac* operon system, expressing the minimal PKS and MAT enzymes



LC-MS analysis of polyketides

• The LC-MS analysis revealed that the minimal PKS + MAT enzymes were able to create polyketide metabolite analogs





LCMS ion extraction of putative product $[M+H]^+$

Conclusions

These experiments show that the native *E.coli* fatty acid MAT domain cannot load the non-native ACP domain OxyD with the malonyl starter unit. Priming of the ACP by the oxytet MAT is necessary for polyketide synthesis. Further research will characterize biochemical MAT-ACP specificity to explain this result.

Acknowledgments

THE BODDY GROUP

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