Heterologous expression of a *Streptomyces clavuligerus* NRPS homologous to indigoidine synthetases

Andriy Kovalchik¹, Ana Ceniceros², Harshwardhan Poddar¹, Marinx H. Medema³, Kirstin Scherlach, Gerrit J Poelarends⁴, Erik Takanov⁵

¹Department of Microbial Physiology, University of Groningen, Nijenborg 7, 9747 AG, Groningen The Netherlands. E-mail: a.ceniceros@rug.nl. ²Groningen Bioinformatics Centre University of Groningen, Nijenborg 7, 9747 AG, Groningen The Netherlands. ³Department of Pharmaceutical Biology, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. ⁴Hans Köl pré Institute Beutenbergstr. 11a, 07745 Jena, Germany, ⁵University of Groningen, Nijenborg 7, 9747 AG, Groningen The Netherlands.

Introduction

1 *Streptomyces clavuligerus*
   - Main producer of clavulanic acid, a β-lactamase inhibitor
   - 48 secondary metabolites clusters found by bioinformatic analysis (Medema et al. 2010; (see figure below)

2 Tautomerases
   - Superfamily of enzymes that catalyze the conversion of a molecule into an isomer that only differs in the position of a hydrogen atom (Poelarends et al, 2006)

   ![Figure showing Aldolase activity](image1.png)

   - The enzyme encoded by this gene contains a C-terminal tautomerase domain uncommon for indigoidine synthetases (Takahashi et al. 2007)
   - indC and indC-trunc (without the tautomerase domain) were cloned into pSET152-ermE⁴ and introduced into *Streptomyces coelicolor* M1146 (\(\Delta\text{act}, \Delta\text{red}, \Delta\text{cda} \Delta\text{cpk}\)), which is an antibiotic non-producer strain (Gomez-Escríban et al. 2011)
   - indC and indC-trunc were introduced into *Escherichia coli*
   - The tautomerase domain of IndC was expressed in *E. coli*

3 RESULTS

1 A gene homologous to this NRPS was discovered in *S. clavuligerus* by bioinformatic predictions (SCLA\_p1474 (indC))

\[\text{DNA} \rightarrow \text{DNA}+\text{100mM Na-glutamate} \rightarrow \text{IndC} \rightarrow \text{Complete IndC} \rightarrow \text{Empty vector}\]

   - The protein encoded by this gene contains a C-terminal tautomerase domain common for indigoidine synthetases (Takahashi et al. 2007)
   - Production of indigoidine in *Streptomyces clavuligerus* M1146 (\(\Delta\text{act}, \Delta\text{red}, \Delta\text{cda} \Delta\text{cpk}\))

2 Addition of glutamate in the media increases the production of indigoidine

   - IndC and IndC-trunc were coexpressed together with a PPTase in *E. coli*. Indigoidine was successful produced (see figure below). This was used further to extract and purify the enzymes to study the function of the extra tautomerase domain.

3 Truncation of the tautomerase domain resulted in a higher production of indigoidine

   - Heterologous expression of IndC and IndC-Trunc in *S. coelicolor* M1146 (\(\Delta\text{act}, \Delta\text{red}, \Delta\text{cda} \Delta\text{cpk}\))

4 Indigoidine was successfully produced (see figure below). This was used further to extract and purify the enzymes to study the function of the extra tautomerase domain.

5 Expression and purification of the tautomerase domain in *E. coli* was successful
   - Tested against all known tautomerase substrates
   - Activity only observed in a non-common tautomerase substrate, p-hydroxyphenyl enolpyruvate, and a weak Michael addition reaction

Conclusions

- The deletion of the tautomerase domain induces a higher production of indigoidine in *Streptomyces*
- The tautomerase domain is not active against normal tautomerase substrates.
- Tautomerase activity could not be confirmed.

References


Future work

- In vitro analysis of the tautomerase domain activity using purified indigoidine.