Transformation of *Saccharopolyspora erythraea* DNA into *Streptomyces lividans* on a high copy plasmid leads to

Activation of a silent antibiotic gene cluster

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INTRODUCTION

Historical perspective

During the "golden age" of antibiotic drug discovery in the 1940's and '50's hundreds of new antibiotics were found from soil-screening programs. This method is no longer cost effective due to the rediscovery of old antibiotics and the low probabilities of finding new antibiotics (Baltz 2005).

However it is becoming increasingly apparent that the majority of actinomycetes have silent antibiotic gene clusters (Bentley 2002). Some clusters can be activated to produce novel biologically active compounds (Laureti 2011). This could mean that the majority of actinomycete antibiotics have yet to be found.

A "product-first" solution

Today most discovery programs are taking a "science-first" approach--attempting to elucidate antibiotic structures from DNA sequence data followed by expression in a heterologous host (Bachmann 2014). This method has been slow to produce new drugs due to the long development time needed to perfect the technology (Jensen 2014). The approach described here is a "product-first" solution, that stresses the generation of new drugs first, followed by reverse engineering to elucidate the science behind the process after the new drugs are found.

METHODS

General cloning materials and methods for *S. lividans* are described by Keiser (2000). The method for this study was to activate the silent actinorhodin gene cluster from *Streptomyces lividans*. Actinorhodin is a blue-pigmented antibiotic and is easily visually identified on agar.

- Cut *Sac. erythraea* DNA into 2-5 Kb fragments.
- Clone into a high copy number plasmid.
- Transform ligation into *E. coli*, harvest plasmid DNA.
- Transform plasmid DNA into *Streptomyces lividans.*
- Visually screen 50,000 transformants.
- Recover plasmids from blue *S. lividans* transformants and identify the activator gene.

RESULTS

SACE_0905

One of the genes from *Sac. erythraea* that activated actinorhodin production in *S. lividans* was SACE_0905. When SACE_0905 was cloned on a high copy number plasmid (pFL1040) in *S. lividans*, the transformants turned a dark blue color. As a control, the parent plasmid pFL8 was also transformed into *S. lividans* (Fig. 1).

Fig. 1. Activation with SACE_0905

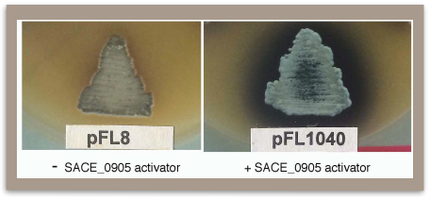
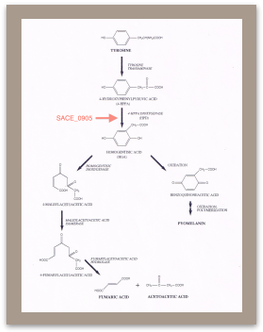


Fig. 2. Tyrosine Catabolic Pathway

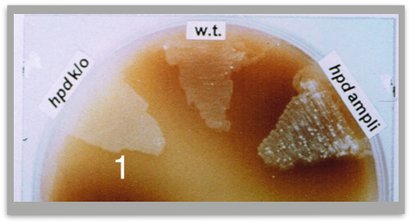


SACE_0905 codes for 4-hydroxyphenylpyruvate dioxygenase, an enzyme in the tyrosine catabolic pathway (Fig. 2). To learn more about why this gene activated actinorhodin production in *S. lividans* we looked at its mutant phenotypes in *Sac. erythraea*. Knockouts of this gene in *Sac. erythraea* were found to be non-pigmented and non-sporulating; and strains carrying additional copies of this gene had increased pigmentation and sporulation (Fig. 3).

AIM

To develop of a natural products drug discovery platform that starts with actinomycetes that are considered to be antibiotic non-producing. It then transforms those strains with novel activator genes, and screens them for antibiotic producers.

Fig. 3. SACE_0905 mutant strains



Left, Sac. erythraea SACE_0905 (hpd) knockout strain, a derivative of the white strain (FL359) that was integratively transformed with the knockout plasmid, pFL1046; Middle sector, wild type strain FL359; Right sector, FL359 transformed with a plasmid (pFL1040) containing the complete SACE_0905 gene. After integrative transformation, the plasmid copy number was amplified.

SACE_0905 over expression in the red strain

The high pigmentation phenotype seen with over expression of SACE_0905 is reminiscent of the *Sac. erythraea* red strain which is also a highly pigmented variant, and is formed by spontaneous mutation from the white strain. When the red variant is grown on minimal (AVMM) agar, a red pigment is produced (Fig. 4A). Interestingly, SACE_0905 knockout mutants in the red strain are clearly blocked in production of this red pigment (Fig. 4B). Therefore, we can postulate that the spontaneous conversion from the white to the red strains results in over expression of SACE_0905. The phenotypes of the white, red and amber strains vary greatly from one medium to another; and the phenotypes on E20A agar are also shown (Fig. 5)

Fig. 4. Red variant k/o strains

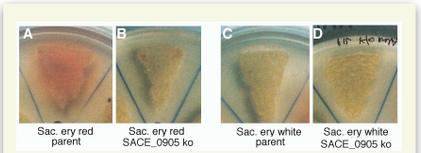


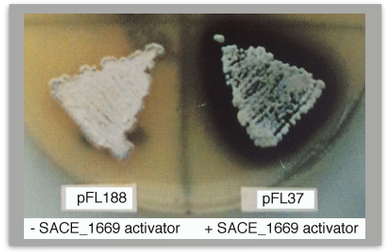
Fig. 5. *Sac. erythraea* phenotypes

Top: White strain, w.t.; Left, red variant; Right, amber variant.

SACE_1669

A second gene from *Sac. erythraea* that was found to activate actinorhodin production in *S. lividans* was SACE_1669 (Fig. 6). The encoded protein has homologies to cell-wall associated peptidases.

Fig. 6. Activation with SACE_1669



Activation of actinorhodin with SACE_1669 was found to be dependent upon the presence of starch in the growth medium. When SACE_1669 was knockedout in *Sac. erythraea* it caused a complete block in pigment production and sporulation on R2T2 agar.

DISCUSSION

In some cases empirical methods for activation of silent antibiotic gene clusters can be easily reverse engineered, for example, when activation of a new natural product is caused by over expression of a pathway specific regulatory gene (Laureti 2011, Ochi 2013). However, the results from this project further illustrate that there are other non-obvious methods for activating silent gene clusters that are not so easily understood. Here we describe two new genes that are activators from *Saccharopolyspora erythraea;* the first is an enzyme of tyrosine metabolism (SACE_0905) and the second is a cell-wall associated peptidase (SACE_1669). Both genes can highly activate actinorhodin production in *S. lividans* when expressed from high-copy plasmids.

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ACKNOWLEDGEMENTS

National Institutes of Health Small Business Innovation Research Award AI34698.

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