Development and optimization of a functional anthracycline biosynthetic pathway in *Escherichia coli* using the BioBrick technique

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MOLECULAR BIOSCIENCES, BIOCHEMISTRY

INTRODUCTION

Natural products and their derivatives are an important source of antibiotics, anticancer agents and other drugs. Most of those are produced by the *Streptomyces* bacteria. Because the production process in *Echerichia coli* is more efficient, it would be beneficial to be able to use it as a host. Even though *E. coli* has been studied as a host for natural products for decades, producing the type II polyketides has been a great challenge.

Natural products called anthracyclines are aromatic type II polyketides assembled by acetyl- and malonyl-CoA units with the help of type II polyketide synthase called minPKS. The minPKS creates a 20-carbon polyketide that is subsequently modified and tailored by a variety of enzymes in order to achieve the aglycone core of anthracyclines.

This study focuses on the early steps of the synthesis pathway and characterization of the early intermediates of nogalamycinone. Nogalamycinone is the aglycone core of an anthracycline antibiotic called nogalamycin. For the purpose of this study, we are creating a new *E. coli* strain, BAP2. A strain called BAP1 has deleted propionate metabolism, so that it won't consume the substrate for polyketides. Because it is also beneficial to minimize the amount of external plasmids in the cell, and the pathway needs help of activators and chaperones to work properly, we are also integrating those genes into the bacterial genome of BAP1 strain.

Using BioBrick method, the nogalamycine pathway is divided into three operons, so that the transcription can be induced in a controlled manner. We will create three different promoters (Xyl/lac, Lux/lac and Las/lac) with specific inducers: xylose, 3-oxo-C6- and 3-oxo-C12-homoserine lactone. IPTG is used as a general inducer for all the operons.

Before the production studies the expression systems are tested with green fluorescent proteins. When the expression system works as desired, the fermentation experiments can be started. The product is analyzed with HPLC and LC-MS. After all this we have created a strain and a method to use for production of anthracyclines so that we have optimized the ratios of proteins in a manner where we get the maximum amount of nogalamycinone and minimal amount of shunt products.

AIMS

1. To create an *E.coli* strain targeted towards optimal anthracycline production
2. Assembly of a functional polyketide biosynthetic pathway in said strain
3. Optomization of the gene expression levels in order to minimize the amount of shunt products and avoid bottlenecks

MATERIALS AND METHODS

1. Choosing the three promoters according to previous studies (Chen *et al.* 2017) and assessing their strength using fluorometry (GFP).
2. Assembly of the expression system in BAP2 *E. coli* strain

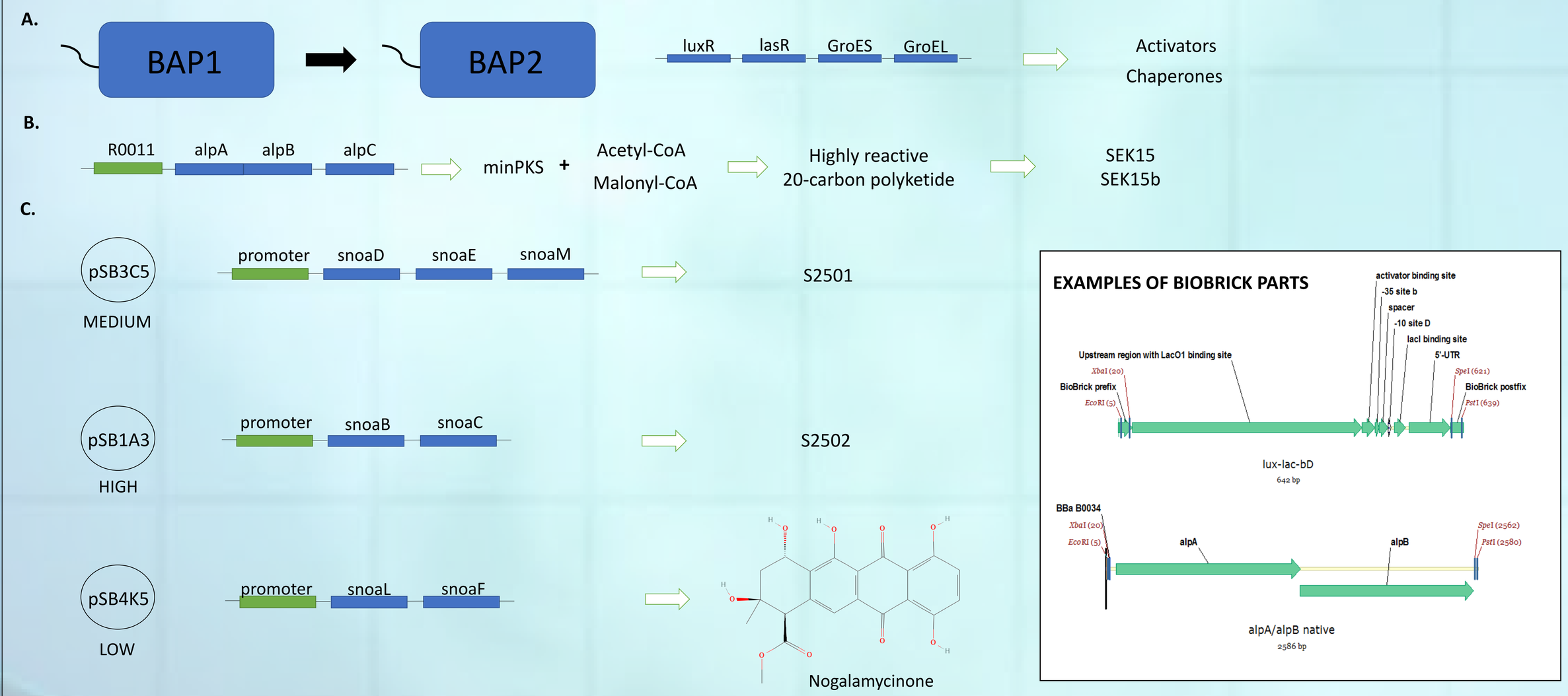
A. Integration of promoter activators and chaperone proteins crucial for the functionality of the pathway into the bacterial genome of BAP1 creating a BAP2 strain.

B. Using standard promoter with the genes leading to minPKS. The highly reactive 20-carbon product is created from acetyl- and malonyl-CoA. If the downstream enzymes are not present, this leads to intermediates called SEK15 and SEK15b.

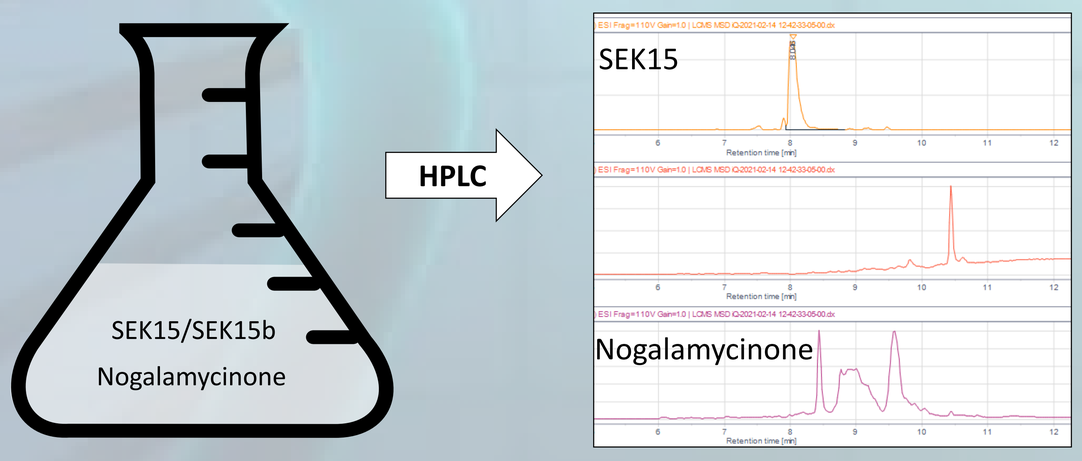
C. Three different vectors and the three different promoter/gene inserts are modified with BioBrick method. This standard method has a circular vector, flanked on the upstream end by EcoR1 and XbaI restriction sites, and on the downstream end by SpeI and PstI restriction sites. Each vector can be cut in four distinct ways yielding four useful fragments. Cutting with EcoRI and SpeI creates a front insert (FI) and cutting with XbaI and PstI creates a back insert (BI). Cutting with EcoRI and XbaI creates a front vector (FV) and cutting with SpeI and PstI creates a back vector (BV). Because of compatible overhangs, back inserts can ligate with back vectors and front inserts with front vectors, resulting to a construct having the same restriction sites as the parent components, with a scar site.

3. When the expression system works as desired, the fermentation experiments can be started. The product is analyzed with HPLC and the mass will be confirmed by LC-MS.

2. ASSEMBLY OF THE EXPRESSION SYSTEM IN BAP2



3. FERMENTATION AND ANALYZING THE PRODUCT WITH HPLC AND LC-MS



RESULTS AND CONCLUSIONS

The promoters have been designed, synthesized and cloned into appropriate vectors alongside the GFP protein. The novel E.coli strain carrying the promoter activators and chaperone proteins crucial for the expression of a fully functional minimal PKS is under development. Immediately after the strain is confirmed to carry all the necessary genes, promoter strength testing will begin. The promoter strength will be measured based on fluorescence. Early intermediates on anthracycline pathways are highly reactive and may lead to formation of shunt products. Therefore correct gene expression levels within a polyketide pathway are highly important. Exact control of gene transcription is not possible in Streptomyces and another benefit of our E. coli approach is that promoters of varying strength are widely available.

In parallel to the promoter development work, we have assembled a pathway for the expression of nogalamycinone in BAP1 + pT-GroE. All gene clusters are expressed under IPTG inducible promoter BBa_R0011 and the products will be confirmed using liquid chromatography and mass spectrometry. This part of the project allows us to assess the gene expression levels and visualize potential products and predict bottlenecks. This part of the project is in its final phase.