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An influence of the copy number of biosynthetic gene clusters on the production level of antibiotics in a heterologous host

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ABSTRACT

Streptomyces albus J1074 is a well-known host for heterologous expression of secondary metabolites. To further increase its potential and to study the influence of cluster multiplication, additional φC31-attachment site was integrated into its genome using a system for transposon mutagenesis. Four secondary metabolite clusters were expressed in strains with different numbers of attachment sites, ranging from one to three copies of the site. Secondary metabolite production was examined and a new compound could be detected, purified and its structure was elucidated.

1. Introduction

Heterologous expression of genes in a suitable host, has been shown to be a powerful tool in biotechnology. Its principle relies on identification of genes and clusters of interest and their expression in a suitable host using optimized expression system. This method is mostly used if the native producer strain is not genetically accessible or shows unreliable growth and metabolite production profiles. Thus it is preferably to express these clusters in a well-studied organism, with a set of available genetic tools and with well described cultivation conditions, media composition and storage conditions. Heterologous expression has already been used to express foreign genes from animals or plants in bacteria or fungi (Frommer and Ninnemann, 1995), to study the function of genes or clusters (Luzhetskyy et al., 2007; Rebets et al., 2015), to elucidate unknown genes or pathways (Baltz 2010), to engineer known or unknown biosynthetic gene clusters (Sánchez et al., 2005; Wenzel et al., 2005), to generate new derivatives of known compounds (Luzhetskyy et al., 2007), to increase the production of desired metabolites (Baltz 2010) or to create unnatural pathways (Gomez-Escribano and Bibb, 2014).

It is also a tool of choice to study secondary metabolite production and is widely used in that field for many purposes. Nowadays streptomycetes are not only a prolific source of new natural products (Baltz, 2008; Hodgson, 2000), but also are suitable hosts for heterologous expression of the secondary metabolites. Several model organisms like *Streptomyces albus* J1074, *Streptomyces coelicolor*, *Streptomyces lividans* or *Streptomyces griseofuscus* have been shown to be fitted for specific tasks whereas also industrial derived strains could be useful for heterologous expression (Baltz, 2010). There are even great efforts undertaken to optimize some of these model organisms to be better hosts (Gomez-Escribano and Bibb, 2011; Komatsu et al., 2010; Komatsu et al., 2013). The advantage in using model organism for heterologous expression is, that these strains are well studied and genetically accessible.

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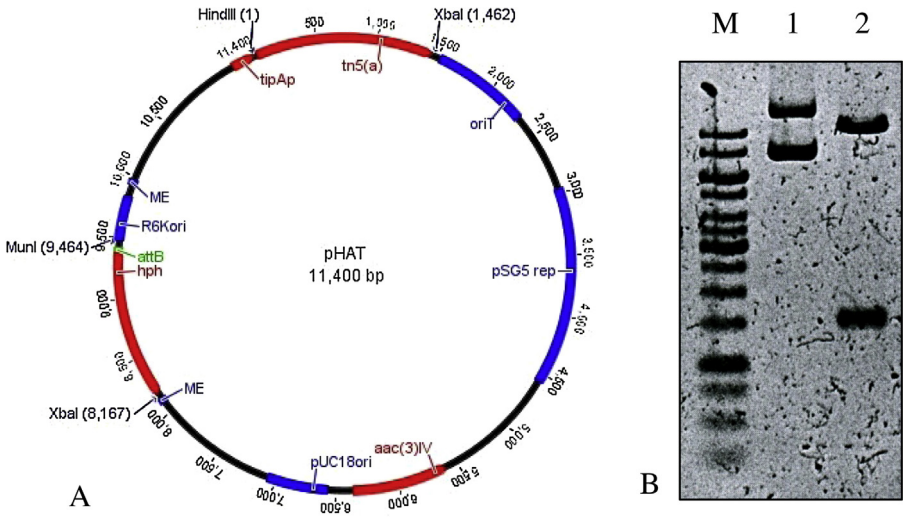

Fig. 1. The map (A) and analytical restriction (B) of pHAT. (A) Plasmid contains following features: oriT—origin of plasmid transfer; pSG5rep—actinomycetes temperature-sensitive replicon; tn5(a)—synthetic transposase gene, under control of tipAp—thiostrepton inducible promoter; aac(3)IV—apramycin resistance marker; hph—hygromycin resistance marker; ME—mosaic end recognition sequence for transposase; R6Kγ-ori—origin for rescue cloning; attB—φC31 phage attachment site. (B) M—1 kb DNA Ladder; 1–undigested plasmid; 2–plasmid digested with HindIII and XbaI. The transposase fragment is visible as 1,5 kbp and the backbone as 10 kbp.

Despite a vast range of available expression hosts, heterologous expression often does not result in the desired outcome. Often the yield of the desired product in the heterologous host is significantly lower than in the parental strain (Binz et al., 2008; Huo et al., 2012). To address this problem, additional steps have to be made, like introducing promoters upstream of the cluster to force its expression or to engineer the precursor supply (Rebets et al., 2015). These steps are quite often not intuitive and very time consuming. Toxicity is also a great issue, because the expression hosts are often more susceptible to the heterologously produced compounds than the natural producers. This problem can be solved by the co-expression of corresponding resistance genes, which also needs time and knowledge about the produced substance.

To address problems as product yield, we integrated additional *attB* attachment sites of the phage φC31 into the genome of our heterologous host *Streptomyces albus* J1074 via Tn5 transposon mutagenesis (Petzke and Luzhetskyy, 2009). The integration system of this phage (Lomovskaya et al., 1970) promotes the recombination between the *attB* site present in the genome of the host and the *attP* site present on the vector (Thorpe and Smith Margaret, 1998). After incorporation of the additional attachment sites into the genome of *S. albus* J1074 we aim to increase the copy number of the integrated gene cluster, and thus to enhance the production of the desired compound.

Here we present a nonspecific approach to increase the product yield of expressed secondary metabolite clusters by addition of *attB* attachment sites in our heterologous host *Streptomyces abus* J1074. We selected several antibiotic clusters and expressed them in a range of *S. albus* mutants with different numbers of *attB* attachment sites to study the effect of gene cluster dosage in these strains. Furthermore we were able to identify a new aranciamycin derivative produced by one of our mutants.

2. Materials and methods

All plasmids, primers and strains used in this work are listed below (Table 1, 2 and 3, respectively).

2.1. *Introduction of additional* attB-*sites into* S. albus-*genome*

In order to construct the plasmid carrying the minitransposons with *attB* the *hph* gene was amplified using pAL1 as a template,

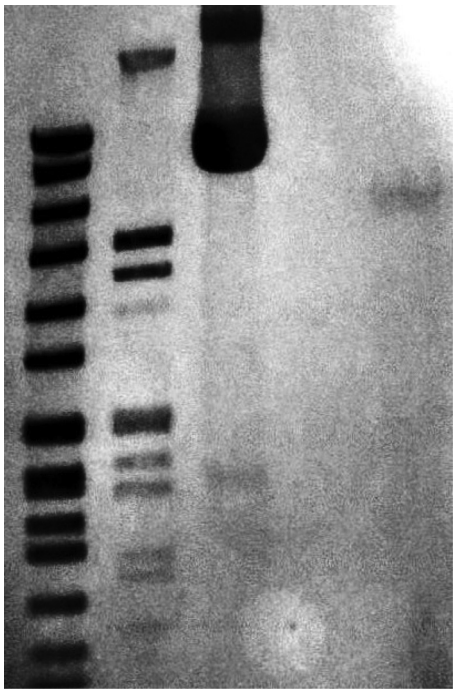

Fig. 2. Southern hybridization of S. albus J1074 with integrated pHAT(II)3. (1) 1 kb DNA ladder; (2) DIG labeled ladder III (Roche); (3) Positive control (pHAT(II)3); (4)Negative control (Chromosomal DNA of S. albus J1074); (5) Chromosomal DNA of S. albus J1074:pHAT(II)3 mutant, T1.

Fr-MI-attB-hph as a forward primer, carrying *attB* and the MunI restriction site, and Rs-XI-hph as a reverse primer carrying the XbaI site. The amplified fragment was cloned into the MunI and XbaI sites of pTn5Oks resulting in pTn5OksattBhph(II). The EcoRV fragment from pTn5OksattBhph(II), containing the transposon, was ligated into linearized by EcoRV pNLTn5, to give pHAT(II)3 (Fig. 1A).

To verify the obtained construct, analytical restriction mapping with EcoRV was performed (Fig. 1B). The obtained 1,9 kb fragment corresponds to the minitransposon construct cloned from pTn5OksattBhph(II).

Additional *attB* attachment sites were introduced by conjugation of the plasmid pHAT(II)3 into the *S. albus* J1074 chromosome