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Making *E. coli* an Erythromycin Production Plant

Tilmann Weber[1,*]
[1]Eberhard-Karls-Universität Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Mikrobiologie/Biotechnologie, Auf der Morgenstelle 28, 72076 Tübingen, Germany
*Correspondence: tilmann.weber@biotech.uni-tuebingen.de
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The production of bioactive compounds in heterologous hosts has become a valuable tool for the investigation of biosynthetic pathways and their rational engineering. In this issue of *Chemistry & Biology*, Zhang et al. (2010) report the manipulation of *E. coli* for the production of erythromycin A.

Many bioactive compounds, including clinically important antibiotics such as erythromycin, are synthesized by microorganisms that often are only poorly accessible by molecular genetic techniques. Thus, genetic engineering of these biosynthetic pathways requires substantial efforts as basic protocols for gene transfer, DNA isolation, and mutagenesis have to be individually developed for each producing strain. Therefore, the expression of complete biosynthetic pathways in heterologous hosts is a promising way to engineer and improve such compounds. Heterologous expression of small gene clusters encoding biosynthesis of aromatic polyketides has already resulted in good yields (see Lopez et al., 2010) and allowed the manipulation of the pathways by standard methodology. Using this approach, derivatives of the natural compounds could be generated in a much easier way, when compared with engineering the original producing strains. Nevertheless, these studies mostly used closely related expression hosts.

However, basic knowledge of growth, cultivation properties, metabolism and physiology, and the availability of molecular tools are scarce in these heterologous production hosts in comparison with the model organism of choice for molecular biology, *Escherichia coli.* Therefore, many efforts have been undertaken to make *E. coli* available as a host for the heterologous production of secondary metabolites (for review, see Gao et al., 2010); thus far, only few success stories have been reported. These include the production of amorpha-4,11-diene, a precursor of artemisinin (Martin et al., 2003), 2,6-dimethyl-3,5,7-trihydroxy-7-(3′-amino-5′-hydroxyphenyl)-2,4-heptadienoic acid, a precursor of rifamycin (Watanabe et al., 2003), epothilone B and C (Mutka et al., 2006) or the production of several fungal and plant polyketides (for review, see Gao et al., 2010).

In 2001, the biosynthesis of 6-deoxyerythronolide B (6-dEB), the macrocyclic core of the antibacterial macrolide erythromycin A, in a rationally engineered *E. coli* strain was described (Pfeifer et al., 2001). The *E. coli* strain BAP1 was constructed via the expression of the *Bacillus subtilis* phosphopantetheinyl transferase Sfp, and BAP1 was now able to attach the phosphopantetheinyl prosthetic group essential for the activity of the erythromycin polyketide synthase. In addition, its metabolism was optimized to provide the 6-dEB building blocks in sufficient amounts by deleting genes involved in propionate catabolism and by introducing genes for propionyl-CoA and *(2S)*-methylmalonyl-CoA biosynthesis. Initially, yields of 20 mg × l^{-1} of the biologically inactive erythromycin precursor 6-dEB were obtained in the BAP1 host, which could be increased by process optimization up to 1.1 g × l^{-1} (Lau et al., 2004).

In the following years, further steps were described to reconstitute the complete erythromycin biosynthetic pathway in *E. coli*. The combination of two tailoring genes and 14 genes involved in deoxysugar biosynthesis and the attachment of the macrolide megalomicin biosynthetic gene cluster in the *E. coli* strain producing 6-dEB resulted in the heterologous production of erythromycin D and erythromycin C at low titers (Peirú et al., 2005). However, with this approach, the heterologous production of the biosynthetic end product erythromycin A had not been achieved.

In this issue of *Chemistry & Biology*, Zhang et al. (2010) report the reconstruction of the erythromycin A biosynthetic pathway of *Saccharopolyspora erythraea* in *E. coli* BAP1. In addition to the erythromycin PKS genes, the authors assembled 17 genes responsible for deoxysugar biosynthesis, macrolide tailoring, and resistance of *S. erythraea* into two operons, placing *eryBI-BVII and ermE* and *eryCI-CVI, eryF, eryG, eryK* under the control of T7 promoters. In a first approach, a two host strategy was applied: the precursor 6-dEB was produced in the previously described

strain expressing the three erythromycin polyketide synthases DEBS1-3 (Pfeifer et al., 2001), extracted, and fed to a second *E. coli* strain expressing the *eryBI-BVII, ermE* and *eryCI-CVI, eryF, eryG, eryK* operons and the chaperon system GroEL/GroES. Clear signals for erythromycin B and D were detected but none for erythromycin A. This indicated that the activity of the P450 hydroxylase gene *eryK*, the last gene in an operon of nine genes, was not expressed, probably due to its position in the operon. After reintroducing a second copy of the *eryK* gene under the control of an extra T7 promoter, erythromycin C and also the bioactive erythromycin A were produced in yields of 2 mg × l^{-1} and 10 mg × l^{-1}, respectively.

When the authors combined expression constructs containing all 20 *S. erythraea* erythromycin biosynthesis genes in a single strain, erythromycin A was detected in titers of 0.6 mg × l^{-1}. Although this yield is still far away from titers obtained by *S. erythraea* industrial producers, which reach up to 7.5 g × l^{-1}, it demonstrates that heterologous expression of complex type I polyketide biosynthesis pathways is feasible in *E. coli.* It is very likely that these titers now can be optimized using standard strain improvement technologies established for *E. coli*.

The authors also succeeded in generating glycosylated erythromycin analogs in the heterologous *E. coli* host by replacing the native erythromycin polyketide synthase genes with engineered variants (Liu et al., 1997; Pfeifer et al., 2001) in titers between 0.05-0.49 mg × l^{-1}. Such further reduction in productivity is frequently observed when engineered biosynthetic genes are expressed in the original producers.

Although it is rather unlikely that *E. coli* will replace industrial production strains for the production of wild-type compounds, it will be undoubtedly an interesting alternative for combinatorial biosynthesis and pathway engineering approaches, as these can be carried out much easier in the heterologous *E. coli* system than in the native hosts or closely related organisms.

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