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Combinatorial Biosynthesis of Polyketides: Generation of Multiple Bioactive Macrolides by Hybrid Polyketide Synthases

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Introduction

Modular polyketide synthases (PKSs) are large multifunctional enzymes that are responsible for the biosynthesis of macrolides and other macrocyclic polyketides whose members have diverse structural and pharmacological properties[1]. These modular PKS assemblies are formed by giant multifunctional enzymes harboring one to many modules that catalyze serial condensations of activated coenzyme-A thioester monomers derived from small organic acids such as acetate, propionate, and butyrate[2-4]. Each module contains distinctive active site domains required for one cycle of polyketide elongation. Active sites required for condensation include an acyltransferase (AT), acyl carrier protein (ACP), and β-ketoacyl synthase (KS). Each condensation cycle results in a β-keto group that undergoes all, some or none of the potential processing steps. Catalytic domains that perform these reactions include a ketoreductase (KR), dehydratase (DH), and enoylreductase (ER). The absence of any b-keto processing domain results in the presence of a ketone, a KR alone give rise to a hydroxyl, a KR and DH generate an alkene, while a KR, DH, and ER combination leads to complete reduction to an alkane. After assembly of the polyketide chain, the molecule typically undergoes cyclization by a thioesterase (TE) domain at the C-terminal of the final module. The linearity between the

catalytic domains present and the structure of its polyketide products makes modular PKSs attractive systems for combinatorial biosynthesis[5, 6]. A number of genetic engineering strategies have been used to generate hybrid PKSs including (1) inactivation, deletion, insertion and substitution of one or more catalytic domains, (2) deletion or exchange of complete modules, and (3) combining complete subunits from heterologous PKS clusters[1, 5, 7, 8].

The pikromycin PKS of S. venezuelae has several remarkable features that make it a powerful system for combinatorial biosynthesis, including the ability to produce both 12- and 14-membered ring macrolides[9]. Recent work has shown that alternative expression of the pikromycin PKS results in the generation of two macrolactone structures[10]. Expression of full length PikAIV (the last module required for heptaketide chain elongation) generates the 14-membered ring macrolactone narbonolide, while expression of an N-terminal truncated form of PikAIV (using the alternative translation start codon 600 amino acid downstream of the normal pikAIV start codon) results in skipping of the final condensation cycle to generate the 12-membered ring macrolactone 10-deoxymethynolide. This unusual nature of the S. venezuelae system represents a potentially useful tool for combinatorial biosynthesis to generate multiple products from a single hybrid modular PKS[11, 12].

Another strength of the *S. venezuelae* system is the presence of two tailoring enzymes that have unusual substrate flexibility (recognizing both 12- and 14-membered ring macrolactones). The existence of only one set of desosamine genes indicates that the DesVII glycosyltransferase can accept both 12- and 14-membered ring aglycones[13]. PikC (a P450 hydroxylase) also can accept both macrolide substrates and is active at two positions of the macrolactone system[14]. Since structural modifications are often critical for biological activity, a current challenge for combinatorial biosynthesis is to develop approaches that lead not

only to novel macrolactones, but ones that provide fully elaborated structures.

Recently a novel combinatorial biosynthetic approaches in *S. venezuelae* using a set of hybrid modular systems based on the pikromycin, tylosin and erythromycin PKSs was reported[15]. These three systems were chosen based on their architectural similarity (e.g. the presence of bimodular PikAI, EryAI and TylGI; two final individual modules including PikAIII/PikAIV and TylGIV/TylGV) as well as the structural attributes of their product profiles. These attributes provided a framework from which to address new questions about molecular recognition between heterologous mono-modular PKSs, as well as to probe the flexibility of the DesVII glycosyltransferase and PikC hydroxylase toward novel substrates.

Results

The plasmid-based replacement of the multifunctional protein subunits of the pikromycin PKS in *S. venezuelae* by the corresponding subunits from heterologous modular PKSs resulted in recombinant strains that produce both 12- and 14-membered ring macrolactones with predicted structural alterations. In all cases, novel macrolactones were produced and further modified by the DesVII glycosyltransferase and PikC hydroxylase leading to biologically active macrolide structures.

Conclusions

These results demonstrate that hybrid PKSs in *S. venezuelae* can produce a multiplicity of new macrolactones that are modified further by the highly flexible DesVII glycosyltransferase and PikC hydroxylase tailoring enzymes. This work demonstrates the unique capacity of the *S. venezuelae* pikromycin pathway to

expand the toolbox of combinatorial biosynthesis and to accelerate the creation of biologically active natural products.

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