INVESTIGATING PROGRAMMING OF FUNGAL PKS-NRPS MEGASYNTHASES VIA CONSTRUCTION OF HYBRIDS

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Polyketides, domain swaps, heterologous expression

Introduction. Polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) catalyse production of an extreme diversity of natural products, many with beneficial biological activities[1]. PKS-NRPS hybrids combine acetate-derived polyketide chains with a range of proteinogenic and nonproteinogenic amino acids[2]. Fungal highly-reducing PKSs and PKS-NRPS hybrids work iteratively, with one set of catalytic domains controlling every extension cycle and the degree of reduction and dehydration[3]. However, their programming is cryptic and poorly understood.

We investigate the programming of fungal iterative PKS-NRPSs by conducting domain swaps between closely related enzymes and heterologous expression of the resultant hybrids. Our objective is to explore the potential to produce novel compounds by construction of non-natural hybrid enzymes.

Methods. Multiple gene constructs were created via homologous recombination in yeast. They were shuttled from yeast to *Escherichia coli* and transferred to an *amyB* expression cassette by Gateway LR recombination. Plasmids were introduced into *Aspergillus oryzae*, the heterologous expression host, by protoplast transformation. Metabolites in crude fungal extracts were analysed by LCMS and structures were determined by NMR.

Results. Tenellin (TEN), a dimethylated pentaketide, and desmethylbassianin (DMB), a monomethylated hexaketide, are produced by the insect pathogen *Beauveria bassiana*. The TEN and DMB biosynthetic gene clusters are structurally identical, with 90% identity at the nucleotide level. Swaps between the PKS-NRPS genes (*ten*S and *dmb*S) located chain-length programming to the *β*-keto reduction (KR) domain and methylation patterning to the *C*-methylation (*C*-MeT) domain[4]. In reciprocal swaps, replacing fragments of *dmb*S with the corresponding *ten*S fragments, hybrid genes were co-expressed with *ten*C, encoding the enoyl reductase from the TEN pathway. The major product resulting from *C*-MeT exchange was the dimethylated hexaketide prebassianin A, with minor amounts of monomethylated hexaketide also observed. Small amounts of pretenellin A resulted from swapping both the the *C*-Met and KR domains, but the KR-only swap did not result in the production of any compounds. Similarly no products resulted from exchanging the entire *C*-MeT-KR region, which was expected to convert the DMBS enzyme to TENS. These results are summarised in Table 1.

Table 1. Effects of substituting regions of the *dmbS* gene with its counterparts from *tenS*. ×: no production

Region(s) exchanged	Compound(s) produced
C-MeT domain	prebassianin A/preDMB A
KR domain	×
C-MeT+ KR domains	pretenellin A
C-MeT- KR region	×

Conclusions. Reciprocal domain swaps confirmed that the *C*-MeT domain controls methylation pattern but lacks full fidelity when placed in a heterologous environment. This means that the *tenS C*-MeT domain can accept longer than usual substrates. Addition of the *tenS* KR to the *C*-Met hybrid resulted in production of dimethylated pentaketide, but in very small titer. Swapping the KR domain alone or as part of the whole *C*-Met–KR region appeared to abolish enzyme activity. The results confirm that rational manipulation of domains of closely related PKS-NRPSs is possible, but outcomes are not as predictable as previous experiments indicated.

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