RECONSTRUCTING BIOSYNTHETIC PATHWAYS IN *ASPERGILLUS ORYZAE*

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Introduction. Heterologous gene expression has provided a successful approach to identifying and characterising individual enzymes involved in secondary metabolism. However, full understanding and exploitation of natural product biosynthesis may require simultaneous expression of numerous genes, including those encoding megasynthases. The size of megasynthase genes can make their manipulation difficult, while sequential introduction of several genes into a foreign host may be restricted by the availability of sufficient selection markers.
Our objective was to develop a molecular toolkit that facilitates the rapid assembly of the genes constituting entire biosynthetic pathways, in one or a few multiple gene expression vectors designed to provide high-level expression in *Aspergillus oryzae*.

Methods. Genes are assembled and/or modified in pEYA, a yeast-*E. coli* shuttle derivative of a Gateway® entry vector. Homologous recombination in yeast is used to join overlapping PCR-generated fragments of the target gene with pEYA linearised between *att*L sites. The multigene expression vector pTYGSarg was derived from pTAex3 [1] by converting it to a shuttle vector, filling the *amy*B expression cassette with a Gateway® destination fragment and inserting an additional three expression cassettes [2]. Three genes can be simultaneously inserted into the latter cassettes by homologous recombination in yeast with the vector plasmid cut downstream of each promoter. Gateway® site-specific recombination is used to fill the remaining expression cassette with a megasynthase- or other gene. Protoplast-mediated transformation introduces the four genes into *A. oryzae* in a single step. Many additional genes can be introduced, up to four at a time, on selection variants of pTYGSarg.

Results. The pathway reconstruction strategy (Figure 1) was used to assemble tenellin (ten) and desmethylbassianin (dmb) biosynthetic pathways from *Beauveria bassiana* in a little over one week. Domain swaps between the ten and dmb megasynthases located chain-length- and methylation programming to the

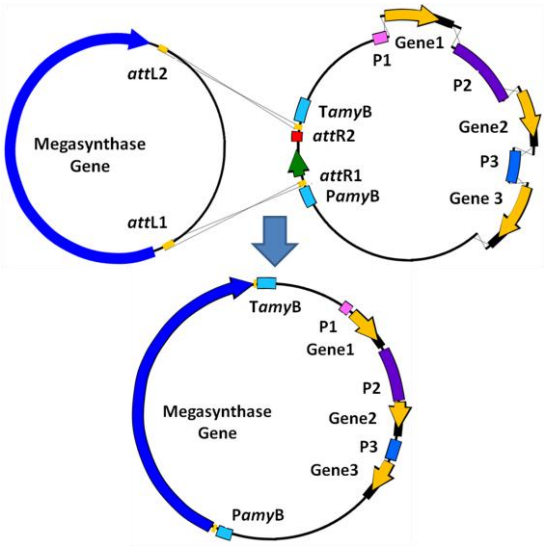

Fig.1 A four-gene biosynthetic pathway reconstructed in a single plasmid (bottom) by homologous recombination in yeast (top right) and Gateway® transfer (top left).

ketoreductase and *C*-methyltransferase domains [3]. Analysis of the aspyridone gene cluster from *Aspergillus nidulans* [4] revealed a non-catalytic role for *apd*B cytochrome P450. Current work focuses on reconstructing pathways for other ten- and dmb-like products, such as militarinone A and torrubiellone A, using gene- and domain swaps to generate novel compounds. Other, non-polyketide pathways are also under construction.

Conclusions. *A. oryzae* is an ideal host for investigating natural product biosynthesis. Pathway reconstruction and introduction are facilitated by the molecular toolkit described.

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