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New approaches for activation of microbial secondary metabolism

Genome sequencing of microorganism revealed that biosynthetic genes involved in secondary metabolites production are arranged in clusters and the majority of these clusters are silent in normal laboratory condition. One way to discover new bioactive natural products is to activate these silent genes either by manipulation of culture conditions or genetic engineering or high throughput elicitor screening (HiTES). In addition to the already available methods, Phenotype guided transposon mutagenesis is a new approach developed by Mohammad R. Seyedsayamdost group, which not only activate a silent gene cluster, but also give a clues about its regulation.

Thailandenes, Cryptic Polyene Natural Products Isolated from *Burkholderia thailandensis* Using Phenotype-Guided Transposon Mutagenesis

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Abstract:

Burkholderia thailandensis has emerged as a model organism for investigating the production and regulation of diverse secondary metabolites. Most of the biosynthetic gene clusters encoded in *B. thailandensis* are silent, motivating the development of new methods for accessing their products. In the current work, we add to the canon of available approaches using phenotype-guided transposon mutagenesis to characterize a silent biosynthetic gene cluster. Because secondary metabolite biosynthesis is often associated with phenotypic changes, we carried out random transposon mutagenesis followed by phenotypic inspection of the resulting colonies. Several mutants exhibited intense pigmentation and enhanced expression of an iterative type I polyketide synthase cluster that we term org. Disruptions of orgA, orgB, and orgC abolished the biosynthesis of the diffusible pigment, thus linking it to the org operon. Isolation and structural elucidation by HR-MS and 1D/2D NMR spectroscopy revealed three novel, cryptic metabolites, thailandene A−C. Variants A and B exhibited potent antibiotic activity against *S. aureus* and *S. cerevisiae* but not against *E. coli*. One of the transposon mutants that exhibited an enhanced expression of org contained an insertion upstream of a σ54-dependent transcription factor. Closer inspection of the org operon uncovered a σ54 promoter consensus sequence upstream of orgA, providing clues regarding its regulation. Our results showcase the utility of phenotype-guided transposon mutagenesis in uncovering cryptic metabolites encoded in bacterial genomes.