Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster

Yunzi Luo, Hua Huang, Jing Liang, Meng Wang, Lu Lu, Zengyi Shao, Ryan Cobb and Huimin Zhao

Abstract

Polycyclic tetramate macrolactams (PTMs) are a widely distributed class of natural products with important biological activities. However, many of these PTMs have not been characterized. Here we apply a plug-and-play synthetic biology strategy to activate a cryptic PTM biosynthetic gene cluster SGR810-815 from *Streptomyces griseus* and discover three new PTMs. This gene cluster is highly conserved in phylogenetically diverse bacterial strains and contains an unusual hybrid polyketide synthase-nonribosomal peptide synthetase, which resembles iterative polyketide synthases known in fungi. To further characterize this gene cluster, we use the same synthetic biology approach to create a series of gene deletion constructs and elucidate the biosynthetic steps for the formation of the polycyclic system. Interestingly, we find that instead of following a sequential reaction order, this single gene cluster may involve three parallel pathways leading to three different products. Similar cluster structure is identified within another PTM biosynthesis gene cluster as well, which indicates that this parallel pathway structure is conserved in PTM biosynthesis. The synthetic biology strategy we employ here bypasses the traditional laborious processes to elicit gene cluster expression and should be generally applicable to many other silent or cryptic gene clusters for discovery and characterization of new natural products.