Establishing a new methodology for genome mining and biosynthesis of polyketides and peptides through yeast molecular genetics

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In this study, we established an innovative approach for biosynthesizing bioactive compounds of fungal origin by focusing on previously uncharacterized biosynthetic gene clusters and using an engineered *Saccharomyces cerevisiae* strain as a surrogate host. We exploited the overlap extension PCR method to quickly synthesize full-length PKS and NRPS genes, which are usually 5- to 20-kb or longer, using a pool of cDNA reverse-transcribed from a total RNA isolated from the source fungus. The amplified gene was subsequently cloned into a yeast expression vector using the recombination capability of yeast. This series of procedures allowed fast and efficient establishment of a yeast system for expression of biosynthetic genes of unknown function and production of corresponding natural products. Our results clearly demonstrated successful expression of four type I iterative polyketide synthase genes and one nonribosomal peptide synthetase gene from three different fungal species in *S. cerevisiae*, all of which led to the production of a total of six compounds whose identities were characterized spectroscopically and verified the speculated functions of these biosynthetic mega-enzymes. Our plasmid-based system provides an advantage over fungal systems in terms of ease and speed of cloning the target genes, and it also tolerates handling of substantially large genes. Also, the use of a plasmid-borne system simplifies the effort of engineering biosynthetic pathways for production of various analogs using traditional molecular biological techniques. By streamlining the process of translating uncharacterized fungal biosynthetic genes into structurally characterized compounds, our methodology should facilitate the efforts in isolating novel natural products and rationally engineering in the biosynthetic pathways for production of analogs possessing comparable or even more potent bioactivity.