Discovery of bioactive microbial metabolites via synthetic biology approaches

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Introduction

Natural products and their synthetic derivatives have been used widely in healthcare, agriculture and other areas [1]. However, the discovery process of natural products has conceptually and technically **not** changed over the past three decades but is only able to find a small fraction (1 to 5%) encoded by natural product operons available in microbial genomes [2,3]. My research group aims to develop both *in vitro* and *in vivo* synthetic biology approaches that allow the thorough exploitation of microbial genomes for the discovery and development of natural products and analogs. Over the past year, we focused on the overproduction of thaxtomin and its analogs using synthetic biology approaches. Thaxtomins are virulence factors of several plant pathogenic *Streptomyces* strains [4] and multiple major agro-chemical companies [5] are developing these bioactive microbial metabolites as a bioherbicide to control weed growth.

Experimental

We refactored thaxtomin gene clusters. The resultant cluster was transformed into *S. albus* and the production of thaxtomin A and other analogs were evaluated with different culture conditions. The titer of thaxtomin A was determined by HPLC. Feeding unnatural building blocks into the culture medium led to the production of unnatural products, whose structures were elucidated by 1D and 2D NMR and LC-MS analyses. In addition, we successfully prepared five recombinant biosynthetic enzymes of thaxtomin (TxtA, B, H, E, and C), and created >120 thaxtomin analogs using these enzymes as determined in LC-MS and NMR analysis.

Results and Discussion

We have achieved the high-yield production of thaxtomins in *S. albus*. The gene cluster from *S. scabiei* 87.22 was cloned and expressed in *S. albus* J1074. LC-MS analysis revealed the production of thaxtomins and nitro-tryptophan analogs in the heterologous host, and screening of fermentation media led to 0.22 g/L of thaxtomins, 22 times higher than the native producer (**Fig.1A**). Additionally, we used the engineered *S. albus* J1074 to produce one unnatural fluorinated analog 5-F-thaxtomin A, whose structure was elucidated by a combination of MS and 1D and 2D NMR analyses. Natural and unnatural thaxtomins demonstrated potent herbicidal activity in radish seedling assays. Some of these results were published in two recent papers [6,7]. Furthermore, we prepared key biosynthetic enzymes TxtA, TxtB, TxtC, TB14, and TxtH of thaxtomins in *E. coli*. Using these enzymes as biocatalysts, we developed multiple on-pot routes that synthesized >180 thaxtomin analogs from 6 tryptophan and 12 phenylalanine analogs (**Fig.1B**). Some of these results were published in a recent paper [8] and one manuscript in review [9].

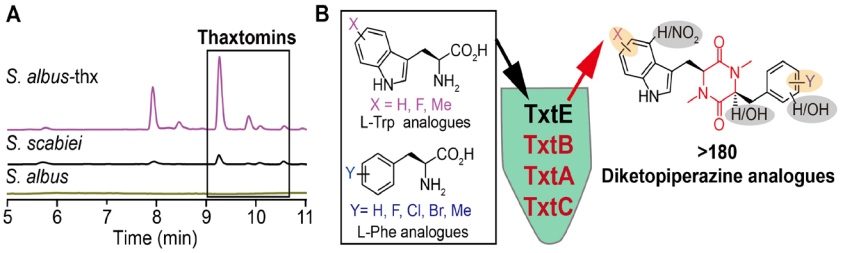

Fig.1 Synthetic biology approaches to the production of thaxtomins. **A:** Heterologous expression of the thaxtomin gene cluster in *S. albus* led to 22-fold improvement of thaxtomin yield compared with the native producer. **B:** *In vitro* synthetic biology approach using four biosynthetic enzymes generated over 180 unnatural diketopiperazine analogues.

Conclusions

In the proof-of-concept study, we demonstrated that both cell-based and –free synthetic biology strategies can be promising to discover and develop natural products and analogs from microbial genomes. Specifically, we improved the titer of thaxtomin by over 22 folds and synthesized hundreds of new analogs.

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