Discovery of Novel Terpenoids from UbiA Terpene Synthases

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The Bacterial Terpenome

Terpenoids comprise the largest, most structurally diverse family of natural products with over 80,000 known members.[1] Yet, only a fraction have been isolated from bacteria; despite many bacterial terpenoids possessing important biological activities ranging from antibacterial, anticancer, and immunosuppressive. For example, the understudied UbiA superfamily is known for products like cyathin Q, which has been reported to be a potent anticancer agent.[2] This study screens predicted UbiA Terpene Synthase (TS) genes in an engineered terpene precursor overproduction system to identify novel terpene scaffolds. The findings expand terpenoid diversity, provide insights into UbiA TS enzymes, and have implications for drug development and industrial applications.

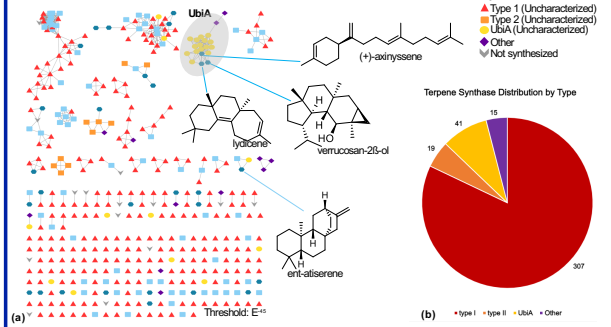


Fig. 1(a) Sequence similarity network of 450 TSs of the Rudolf Lab. Refer to key **(b)** Terpene synthase distribution by type.

Terpene Precursor Overproduction System (MKI4)

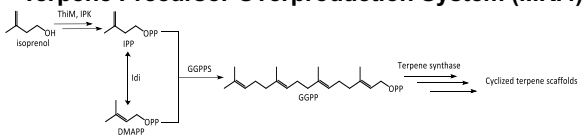


Fig. 2: Biosynthetic pathway for diterpene production with GGPP precursor.

- Genes that code for TSs were cloned into pET-44b plasmids of BL21 Star *E. coli* protein production variant.
- Plasmids were transformed into host
- Antibiotics were used to select for cells that engulfed the plasmid
- Surviving cell colonies were transformed into TB media and allowed to grow overnight

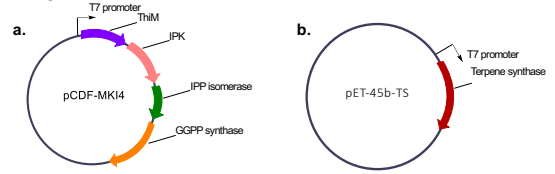


Fig. 3. a) Plasmid showing locations of IPK, and ThiM that catalyzes the transformation of isoprenol into DMAPP and IPP and GGPP synthase that transforms the isomers into GGPP precursors for diterpene production, **b)** Low-copy number plasmid for expression of membrane-bound UbiA proteins in the MKI4 system that has the TS gene.

High-Performance Liquid Chromatography Analysis

Screened	System	Hits	Known TS hits
41	MKI4	10 (~ 24%)	2

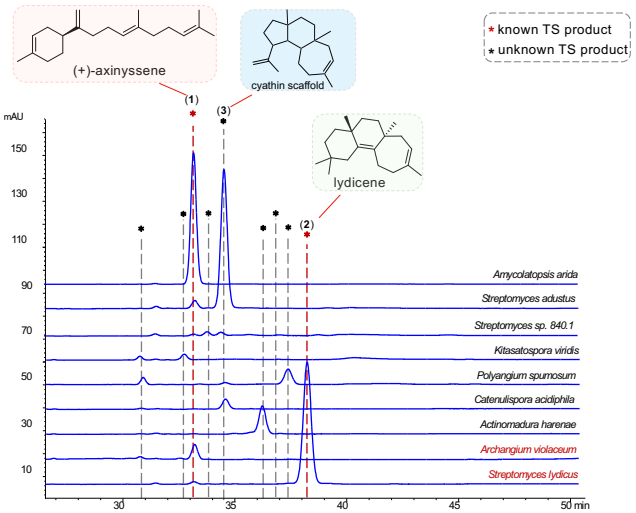


Fig. 4: Analytical HPLC results from UbiA TS screening. Only results that yielded new peak(s) are shown. Compound 3 is known but has been only extracted from sponge. Wavelength for this HPLC trace is 210 mAU

Proposed Biosynthetic Pathway

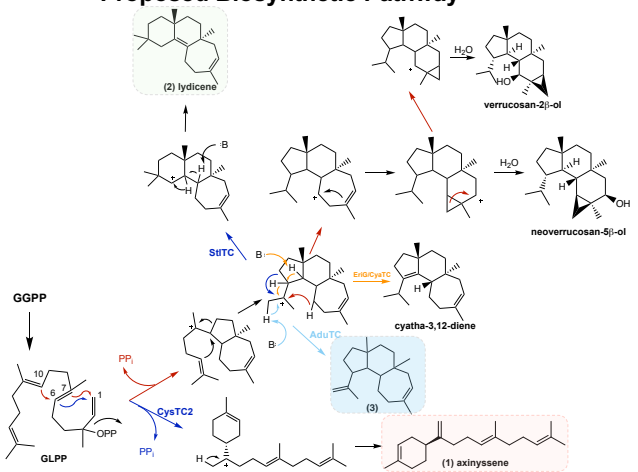


Fig. 5: Proposed mechanism for the formation of axinyssene, cyatha-3,12-diene, and lydicene from GGPP precursor catalyzed by UbiA diterpene cyclases. [2]

Sequence Alignment

Fig. 6: Protein sequence alignment of axinyssene and cyathin-producing enzymes revealed multiple highly conserved residues including two acidic motifs typical of a diterpene cyclase enzyme: **NQxxxxxED** and **DxxDxxxD**. *Hericium erinaceus* and *Cyathus africanus* are fungal proteins that both produce cyatha-3,12-diene.

Docking and Mutation

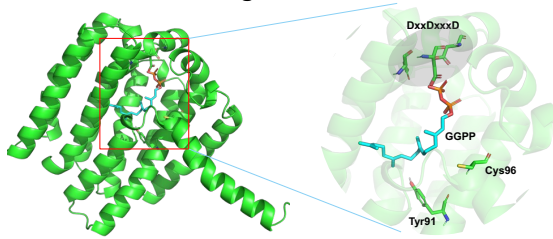
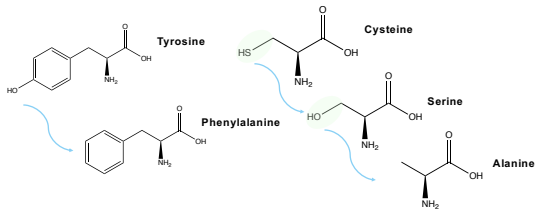


Fig. 7: *Streptomyces adustus* TS with GGPP docked into as ligand. Mutation sites at Tyr91 and Cys96 were selected. Protein shows a DxxDxxxD acidic motif which is typical of diterpenoid enzymes



Tyrosine (Tyr) and Cysteine (Cys) both can serve as bases to help terminate cyclization. Mutating Tyr91 to Phenylalanine (Phe) will ensure that the OH group is lost; whilst maintaining the aromatic side ring that uses π-cation interactions to stabilize transient cations. Likewise, changing Cys96 to Serine will reduce the pKa of the residue affecting how cyclization quenches. Further mutating Cys96 to Alanine will ensure residue losses its basic property.

Future Directions

- Dock compound 3 and other previously isolated compounds from Fig 5.
- Select residues close to scaffolds and target them for mutation.
- Screen mutants and characterize products isolated

References and Acknowledgments

1. Rudolf, J. D.;* Alsup, T. A.; Xu, B.; Li, Z. *Nat. Prod. Rep.*, 2.
2. Zhang, S., Ma, K., Xu, Y., Tao, Q., Chen, Y., Chen, J., Guo, S., Ren, J., Wang, W., Tao, Y., Yin, B., & Liu, H. (2017). Discovery and Characterization of a New Family of Diterpene Cyclases in Bacteria and Fungi. *Angewandte Chemie International Edition*, *56*(17), 4749-4752.

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