JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Review

Secondary Metabolic Potential of *Kutzneria*

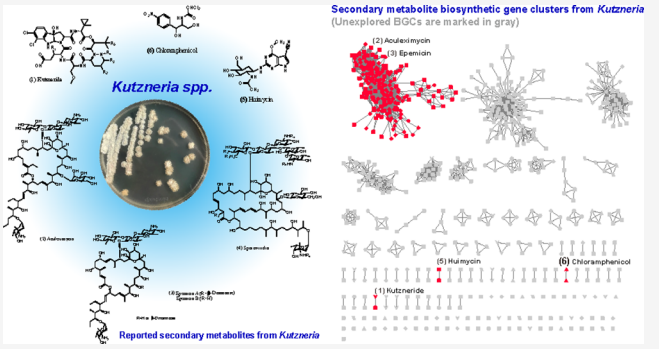
Bin Wei, Ao-Qi Du, Ti-Ti Ying, Gang-Ao Hu, Zhen-Yi Zhou, Wen-Chao Yu, Jing He, Yan-Lei Yu, Hong Wang,* and Xue-Wei Xu*

Cite This: *J. Nat. Prod.* 2023, 86, 1120−1127

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: *Kutzneria* is a rare genus of Actinobacteria that harbors a variety of secondary metabolite gene clusters and produces several interesting types of bioactive secondary metabolites. Recent efforts have partially elucidated the biosynthetic pathways of some of these bioactive natural products, suggesting the diversity and specificity of secondary metabolism within this genus. Here, we summarized the chemical structures, biosynthetic pathways, and key metabolic enzymes of the secondary metabolites isolated from *Kutzneria* strains. In-depth comparative genomic analysis of all six available high-quality *Kutzneria* genomes revealed that the majority (77%) of the biosynthetic gene cluster families of *Kutzneria* were untapped and identified homologues of key metabolic enzymes in the putative gene clusters, including cytochrome P450s, halogenases, and flavin-dependent *N*-hydroxylases. The present study suggests that *Kutzneria* exhibits great potential to synthesize novel secondary metabolites, encodes a variety of valuable metabolic enzymes, and also provides valuable information for the targeted discovery and biosynthesis of novel natural products from *Kutzneria*.



INTRODUCTION

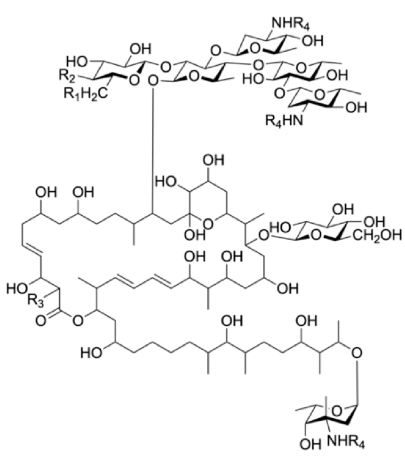
Actinomycete strains are known as an important source of bioactive natural products, especially *Streptomyces*, which account for about 75% of secondary metabolites produced by actinomycetes.[1] Recent studies indicated that some rare actinomycetes, such as *Kutzneria*, *Kibdelosporangium*, *Kitasatospora*, and *Actinosynnema*,[2−6] contain many biosynthetic gene clusters (BGCs) and produce many unique active natural products. The genus *Kutzneria* was proposed by Stackebrandt et al. in 1994[7] and currently only comprises 10 species. *Kutzneria albida* DSM 43870 is the first species in the genus *Kutzneria* with a completely sequenced genome,[8] and only nine genome sequences of strains in this genus are publicly available. Our recent study indicated that the average number of BGCs in *Kutzneria* strains was larger than 30, and the strains showed great individual variation in terms of the total number of BGCs.[2] Recent efforts have identified many bioactive metabolites from *Kutzneria* strains, including some primary (phenolics, fatty acid methyl esters, etc.) and secondary metabolites (depsipeptides, macrolides, etc.).[4,9,10] The biosynthetic pathways of some bioactive secondary metabolites and the metabolic enzymes involved in the biotransformations of these compounds have also been characterized.[4,11−13]

In this review, we will focus on dissecting the secondary metabolic potential of *Kutzneria* through a literature review and in-depth comparative genomic studies. The chemical structures, biosynthetic pathways, and key metabolic enzymes of so far identified secondary metabolites from *Kutzneria* strains will be collected from the literature and presented in a structured manner. The underexplored secondary metabolic potential of *Kutzneria* will be comprehensively investigated based on published genome data using multiple bioinformatic approaches. Besides, the potential valuable metabolic enzymes will be identified.

DISCOVERED SECONDARY METABOLIC POTENTIAL OF *KUTZNERIA*

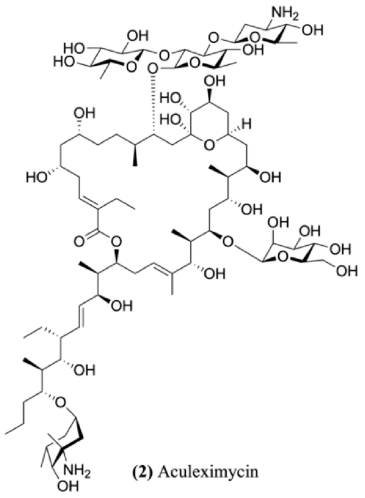
Reported Secondary Metabolites from *Kutzneria*. Sporaviridins (**1**, SVDs) were the first class of secondary metabolites isolated from *Kutzneria* (*K. viridogriseum* DSM 43850, formerly *Streptosporangium viridogriseum* nov. sp.), and they consist of six compounds with each having a 34-membered macrocyclic lactone, one heteropentasaccharide, and two monosaccharides (Figure 1).[14,15] The six SVDs could be converted to the corresponding *N*-acetylated SVDs (*N*-Ac-SVDs) by acetylation in MeOH. All six SVDs showed excellent antimicrobial activity with MIC values less than 1.56 μg/mL against Gram-positive bacteria, including a variety of *Staph-*

Received: January 3, 2023
Published: March 13, 2023

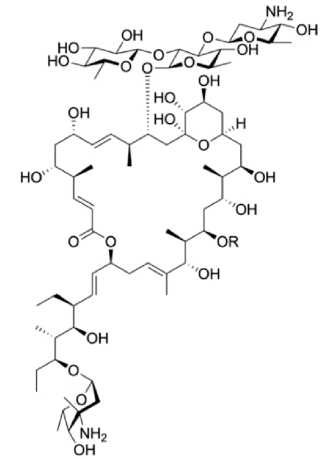


(1) Sporaviridin

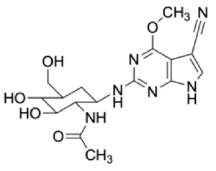
	R_1	R_2	R_3	R_4
SVD-A_1	H	OH	C_2H_5	H
SVD-A_2	H	OH	CH_3	H
SVD-B_1	H	NH_2	C_2H_5	H
SVD-B_2	H	NH_2	CH_3	H
SVD-C_1	OH	OH	C_2H_5	H
SVD-C_2	OH	OH	CH_3	H
N-AC-SCD-A_1	H	OH	C_2H_5	Ac
N-AC-SCD-A_2	H	OH	CH_3	Ac
N-AC-SCD-B_1	H	NHAc	C_2H_5	Ac
N-AC-SCD-B_2	H	NHAc	CH_3	Ac
N-AC-SCD-C_1	OH	OH	C_2H_5	Ac
N-AC-SCD-C_2	OH	OH	CH_3	Ac



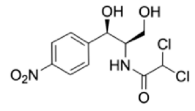
(2) Aculeximycin



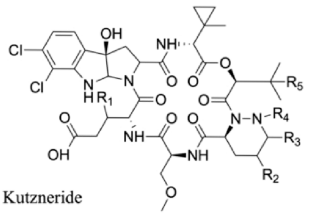
(3) Epemicin A (R=β-D mannose); Epemicin B (R=H)



(4) Huimycin



(5) Chloramphenicol



(6) Kutzneride

	R_1	R_2	R_3	R_4	R_5
Kutzneride 1	HO	H	H	H	CH_3
Kutzneride 2	HO	Cl	H	H	CH_3
Kutzneride 3	HO	H	H	H	CH_3
Kutzneride 4	HO	H	π-bond		CH_3
Kutzneride 5	HO	H	H	H	H
Kutzneride 6	HO	OH	π-bond		CH_3
Kutzneride 7	HO	H	H	H	H
Kutzneride 8	HO	Cl	H	H	CH_3
Kutzneride 9	HO	H	π-bond		CH_3

Figure 1. Chemical structures of secondary metabolites isolated from *Kutzneria* strains.

ylococcus aureus strains, *Streptococcus pyogenes* NY-5, *Enterococcus faecalis* 1501, and *Bacillus subtilis* ATCC 6633.[15] Aculeximycin (**2**) is also a highly glycosylated macrolide and was first isolated from the culture broth of *K. albida* DSM 43870 (formerly *Streptosporangium albidum*) in 1983.[16] Aculeximycin consists of a 30-membered macrocyclic lactone, one trisaccharide aculexitriose, and two monosaccharides (D-mannose and L-vancosamine) (Figure 1).[8] Aculeximycin not only exhibits potent larvicidal activity against mosquito larvae but also showed significant antimicrobial activities against both bacteria and fungi with MIC values less than 3.13 μg/mL.[16] Epemicins A and B (**3**), recently discovered from *Kutzneria* sp. CA-103260, are structurally related to aculeximycin and also contain a 30-membered macrocyclic lactone and the same trisaccharide aculexitriose attached at position C-11. Epemicins A and B exhibit significant antimicrobial properties against methicillin-resistant *Staphylococcus aureus* MB5393 with MIC values less than 4 μg/mL, but they do not affect the growth of four Gram-negative bacteria.[4] Huimycin (**4**) is a new pyrrolopyrimidine natural product recently isolated from *K. albida* DSM 43870.[17] It contains a unique cyano group attached at position C-7 and an *N*-acetylglucosamine moiety.

Kutzneria strains also produce several chlorine-containing natural products. For example, chloramphenicol (**5**) was originally discovered from *Streptomyces venezuelae* ISP5230 in 1947 and was also isolated from *K. kofuense* DSM 43851 (formerly *Streptosporangium viridogriseum* var. *kofuense*) in 1973.[18] Chloramphenicol is a chlorine-containing nitrobenzene and has been widely used as an antibiotic (conjunctivitis, meningitis) since the 1950s.[19] Kutznerides (**6**) are a group of chlorine-containing cyclic hexadepsipeptides, and currently nine related compounds (kutznerides 1−9) have been isolated from *Kutzneria* sp. 744.[11] Kutznerides are composed of five nonproteinogenic amino acids (chlorinated piperazic acid, 2-(1-methylcyclopropyl)glycine, piperazic acid, *O*-methyl-L-serine, 3-hydroxy-D-glutamic acid) and one hydroxy acid (2-hydroxy-3-methylbutyric or 2-hydroxy-3,3-dimethylbutyric acid).[10] Kutznerides 2 and 8 exhibit the highest antimicrobial activity (MIC = 6 μM).[20]

Characterized Secondary Metabolic Pathways and Enzymes from *Kutzneria*. The biosynthetic pathways and key metabolic enzymes for secondary metabolites isolated from *Kutzneria* strains have attracted increasing attention and thus have been partially characterized in recent years. The biosynthetic gene cluster (*epm*) for epemicins A and B (**3**) was identified through heterologous expression and gene inactivation from *Kutzneria* sp. CA-103260.[4] The *epm* BGC is approximately 130 kb and encodes six type I polyketide synthases that are responsible for synthesizing the backbone of epemicins A and B (Figure 2A). The TE domain in EpmP6 is responsible for the cyclization and release of the synthesized backbone. Moreover, several postcyclization enzymes are

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127

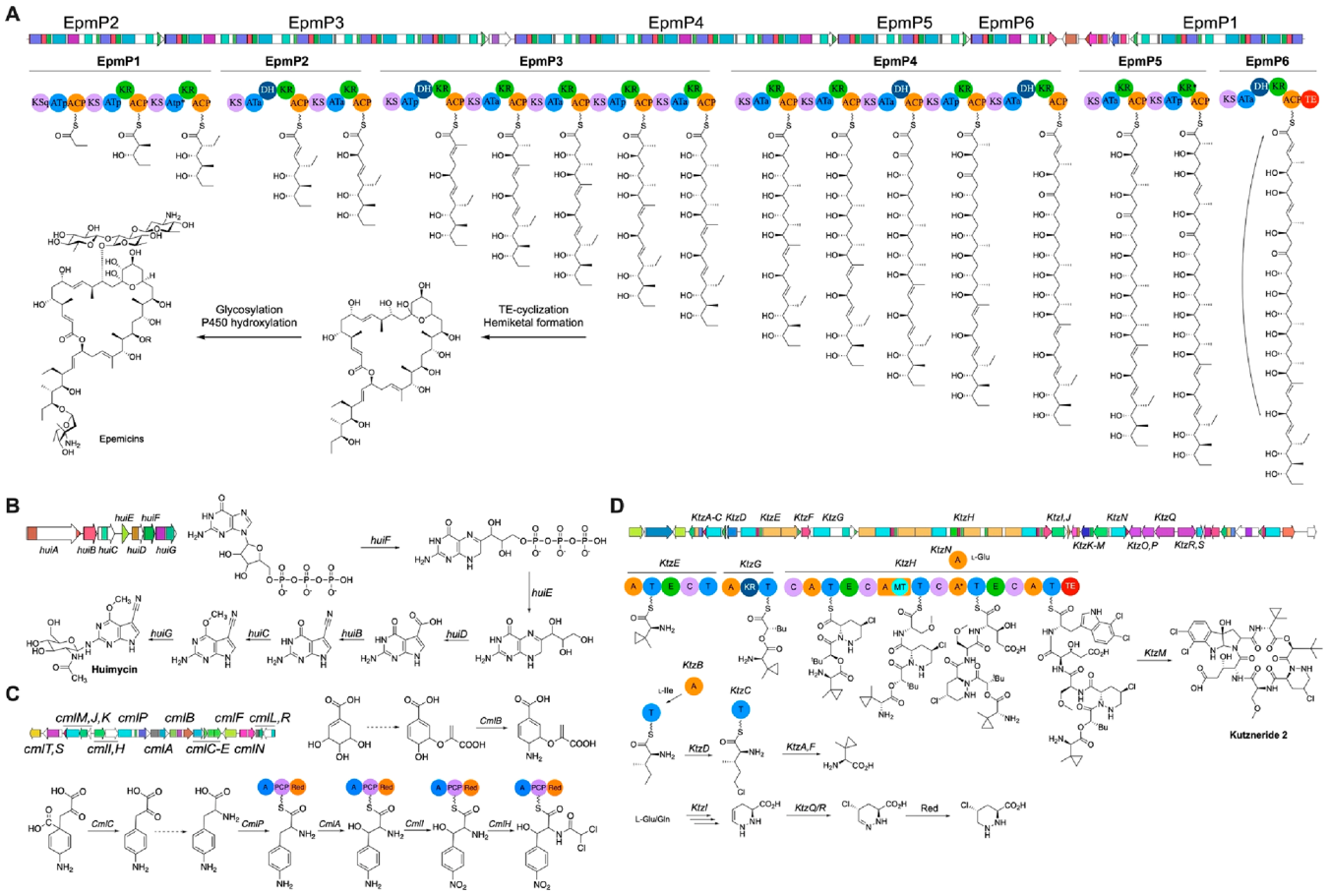


Figure 2. Partially characterized biosynthetic pathways of secondary metabolites isolated from *Kutzneria* strains. (A) Epemicins. (B) Huimycin. (C) Chloramphenicol. The pathway in *Streptomyces venezuelae* has been partially characterized but remains unknown in *K. kofuense* DSM 43851. (D) Kutzneride 2.

involved in the biosynthesis of epemicins, such as the cytochrome P450 protein (encoded by EpmD) and glycosyltransferases (encoded by *epmG1*−epmG6).

The biosynthetic gene cluster (*hui*) encoding the pyrrolopyrimidine huimycin (**4**) was recently identified through heterologous expression and a series of gene deletion experiments in *K. albida* DSM 43870.[17] The *hui* BGC contains at least seven protein-coding genes (*huiA*−*huiG*) (Figure 2B), five of which show high sequence similarity to the corresponding genes within the BGC of toyocamycin (a 7-cyano-7-deazaguanine derivative). The other two genes (*huiC* and *huiG*) encode a SAM-dependent methyltransferase and a glycosyltransferase, respectively, which are likely responsible for the conversion of 7-cyano-7-deazaguanine to huimycin. However, the functions of these proteins have not been experimentally validated.

Chloramphenicol (**5**) has been discovered in a variety of actinomycetes, such as *S. venezuelae* ISP5230, *S. phleochromogenes* var. *chloromyceticus*, and *K. kofuense* DSM 43851.[19,21] The gene cluster (*Cml*) for the biosynthesis of chloramphenicol has been experimentally characterized in *S. venezuelae* ISP5230,[22] which contains at least 17 protein-coding genes (*CmlA*−*CmlT*) (Figure 2C), and the biosynthetic pathway can be generally divided into three steps: shikimate pathway, conversion of shikimic acid to *p*-amino phenylalanine (PAPA), and conversion of PAPA to chloramphenicol.[23] However, the chloramphenicol gene cluster in *K. kofuense* DSM 43851 remains unknown.

Kutznerides (**6**) are a group of cyclic depsipeptides that consist of six nonproteinogenic residues. The gene cluster for kutznerides was identified by degenerate PCR for halogenating enzymes. The *Ktz* BGC spans approximately 56 kb and encodes six nonribosomal peptide synthetase modules that are distributed in three proteins (encoded by *KtzE*, *KtzG*, and *KtzH*) (Figure 2D). The *Ktz* BGC consists of 29 protein-coding genes, and the functions of enzymes encoded by 11 genes (*KtzA*−*D*, *KtzG*−*KtzI*, *KtzN*, *KtzQ*, *KtzR*, and *KtzT*) have been experimentally confirmed.[11,12,24−27] Another five genes are presumed to be involved in the biosynthesis of kutznerides. At least nine tailoring enzymes are identified in the gene cluster, which are mainly responsible for the biosynthesis of the uncommon amino acids; for example, the formation of 2-(1-methylcyclopropyl)-D-glycine involves the following genes: *KtzA*, *KtzC*, and *KtzD*.

Currently, only 14 enzymes or proteins have been functionally characterized from *Kutzneria* strains; 12 of them are derived from *Kutzneria* sp. 744, and the other two enzymes are sourced from *K. albida* DSM 43870 (Table 1). The adenylation domain activities of KtzB, KtzG, and KtzN were investigated by Fujimori et al.,[11] which provided direct biochemical evidence for the biosynthetic pathway of the kutznerides. KtzD is identified as an Fe^{II}-dependent halogenase and cooperates with KtzA−C to generate the novel amino acid 2-(1-methylcyclopropyl)glycine. KtzH is a KtzJ-dependent methyltransferase and plays an essential role in the formation of *O*-methyl-L-serine for kutzneride biosynthesis.[25] KtzT is the

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127

Table 1. Characterized Enzymes or Proteins from *Kutzneria* Strains

enzyme	function	accession no.	source	ref
KtzB	adenylation domain	ABV56582.1	*Kutzneria* sp. 744	11
KtzG	adenylation domain	ABV56587.1	*Kutzneria* sp. 744	11
KtzN	adenylation domain	ABV56594.1	*Kutzneria* sp. 744	11
KtzQ	flavin-dependent tryptophan halogenase	ABV56597.1	*Kutzneria* sp. 744	12
KtzR	flavin-dependent tryptophan halogenase	ABV56598.1	*Kutzneria* sp. 744	12
KtzA	acyl-CoA dehydrogenase	ABV56581.1	*Kutzneria* sp. 744	24
KtzC	carrier protein	ABV56583.1	*Kutzneria* sp. 744	24
KtzD	Fe^{II}-dependent halogenase	ABV56584.1	*Kutzneria* sp. 744	24
KtzH	serine activation and *O*-methylation	ABV56588.1	*Kutzneria* sp. 744	25
KtzT	nitrogen−nitrogen linkage for piperazate	QNJ99246.1	*Kutzneria* sp. 744	26
KtzI	flavin-dependent *N*-hydroxylase	EU074211.1	*Kutzneria* sp. 744	27
KthP	halogenase	KUTG_08892[a]	*Kutzneria* sp. 744	28
KaPH1	2-oxoglutarate-dependent dioxygenase	WP_025358137.1	*K. albida* DSM 43870	29
KalbTG	transglutaminase	AHI00814.1	*K. albida* DSM 43870	30

[a]UniProtKB accession number, and others are NCBI accession numbers.

first characterized enzyme that catalyzes N−N bond formation in natural product synthesis and is involved in the biosynthesis of a building block for the kutznerides.[26] KtzI is a flavin-dependent *N*-hydroxylase that catalyzes the formation of the uncommon nonproteinogenic residue L-N^5-hydroxyornithine.[27]

Apart from the 11 enzymes necessary for kutzneride biosynthesis described above, KthP was identified as a fourth halogenase from the upstream of the *Ktz* BGC that acts on the chlorination of the piperazate residue in kutzneride biosynthesis.[28] KaPH1 is characterized as a highly selective and efficient 2-oxoglutarate-dependent dioxygenase from *K. albida* DSM 43870, which catalyzes the conversion of L-proline to *trans*-4-hydroxy-L-proline with 92.8% yield.[29] KalbTG is a highly site-specific transglutaminase from *K. albida* DSM 43870. It shows no cross-reactivity with commonly used substrates but a high affinity for the motifs YRYRQ and RYESK.[30]

UNDEREXPLORED SECONDARY METABOLIC POTENTIAL OF *KUTZNERIA* SPECIES

The structure-specific secondary metabolites and functionally diverse metabolic enzymes derived from *Kutzneria* strains have prompted us to comprehensively investigate the secondary metabolic potential of *Kutzneria* species. All nine available *Kutzneria* genomes were retrieved and subjected to extensive bioinformatic analysis. Except for two genomes (*Kutzneria* sp. CP_BM_RX_R8_31 and *Kutzneria* sp. CP_BM_ER_R8_23) derived from metagenomes, the other seven genomes have a comparable size (9.8−12.0 Mb) and gene content (8841−10,975), as well as a similar GC content (69.4−70.7%) (Figure

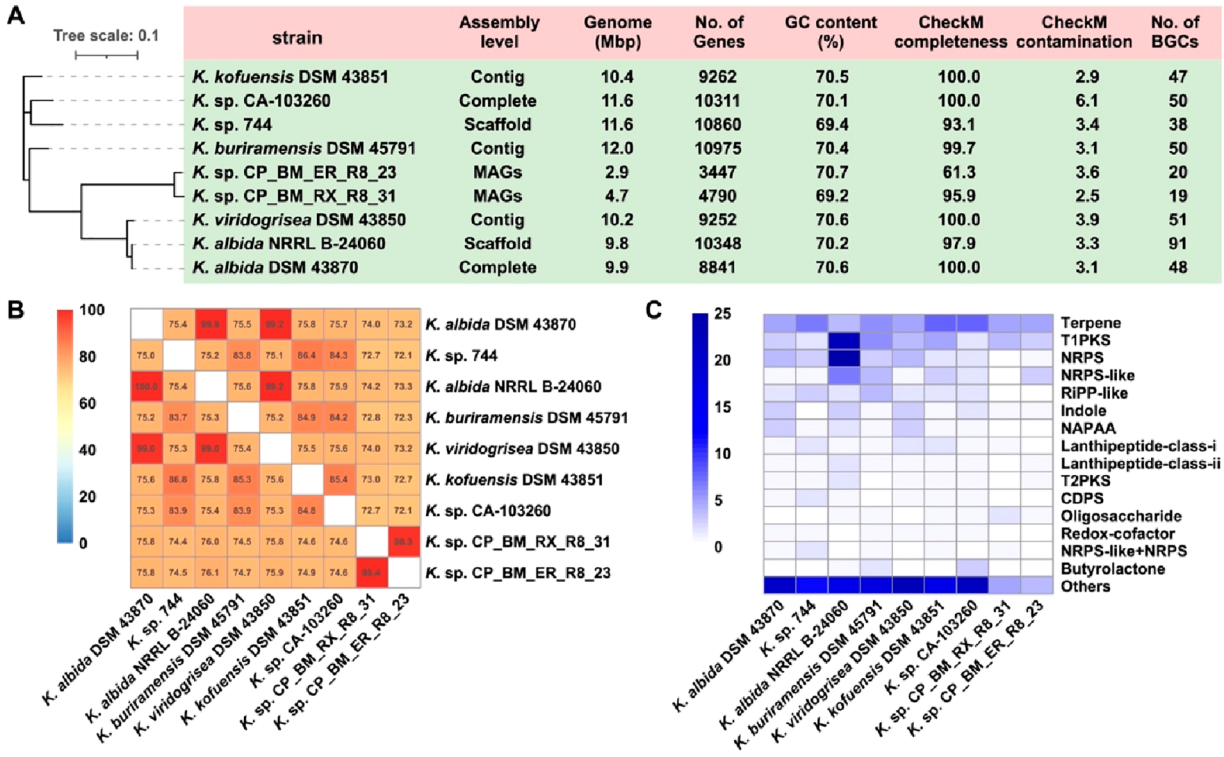


Figure 3. (A) Genomic features of nine *Kutzneria* genomes. The phylogenetic tree was constructed using the UBCG based on the 92 concatenated core gene sequences. (B) Genomic similarities between the nine *Kutzneria* genomes measured by the pattern similarity score. (C) The numbers of different classes of BGCs in *Kutzneria* genomes. RiPPs: ribosomally synthesized and post-translationally modified peptides; NAPAA: non-alpha poly amino acids; CDPS: cyclic dipeptide synthase.

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127

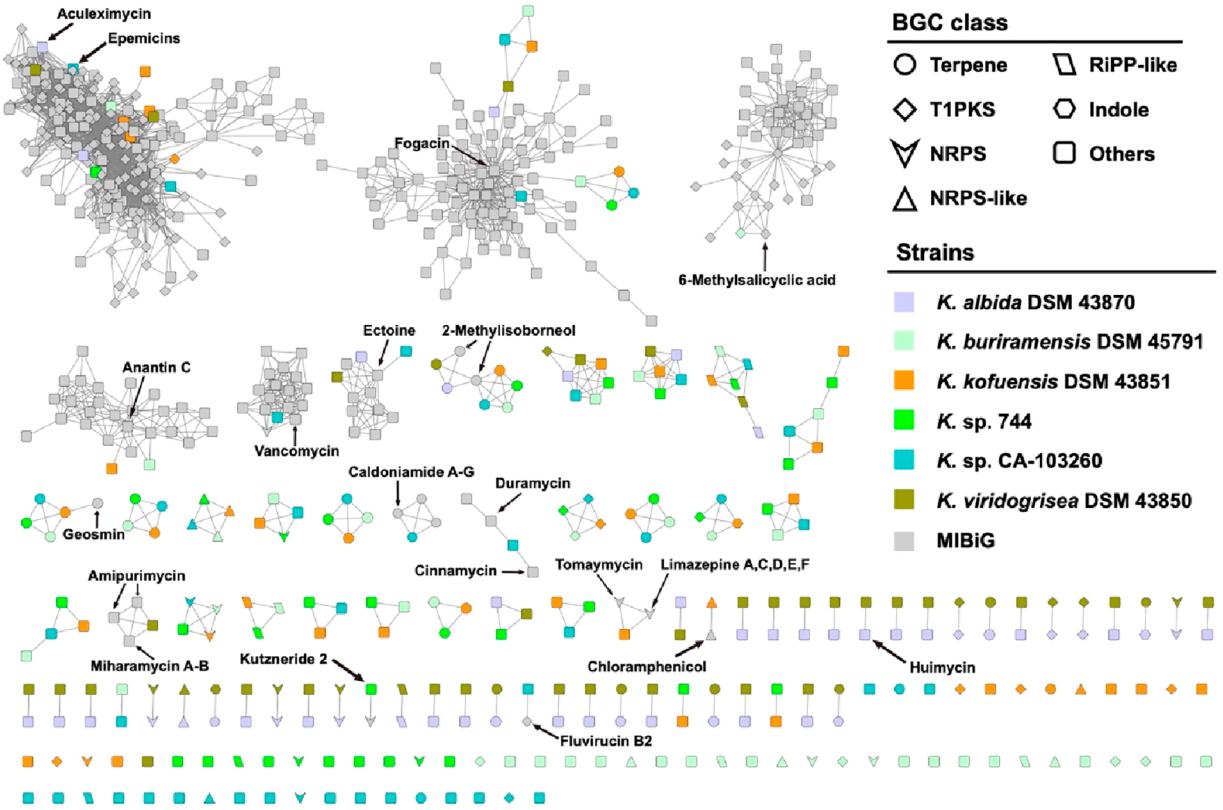


Figure 4. Sequence similarity network of predicted biosynthetic gene clusters (BGCs) from six high-quality *Kutzneria* genomes. Each node represents one BGC, and the gray symbols represent reference BGCs in the MiBIG database. All BGCs presented in this figure are summarized in Table S2.

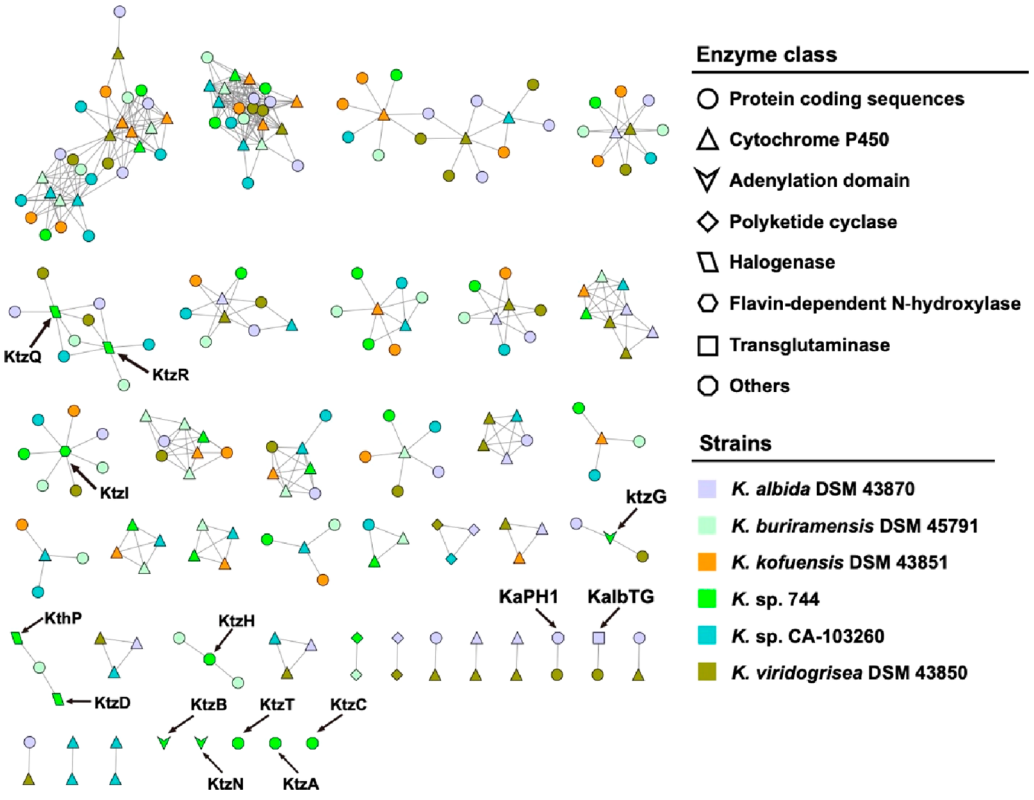


Figure 5. Sequence similarity network of characterized enzymes and their homologous proteins from *Kutzneria* strains. Each node represents one enzyme or protein. All protein-coding genes presented in this figure are summarized in Table S3.

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127

3A). The CheckM results demonstrate that most of the genomes have a high assembly quality with an estimated completeness larger than 93.1% and an estimated contamination less than 6.1%. *K. viridogrisea* DSM 43850 shows a high genomic similarity (>99%) to *K. albida* DSM 43870 and *K. albida* NRRL B-24060, and the numbers of predicted BGCs in these genomes range from 48 to 91 (Figure 3B), while the two metagenome-assembled genomes show high genomic similarity to each other and are predicted to encode 19 or 20 BGCs, suggesting that a low genome assembly quality may underestimate the predicted number of BGCs. The numbers of each class of BGCs in these *Kutzneria* genomes are presented in Figure 3C and Table S1, demonstrating that terpene, type 1 PKS (T1PKS), and NRPS are the most predominant classes of BGCs, and the strains harbor a great variety of BGCs, as many BGCs are classified as Others. Interestingly, *K. albida* NRRL B-24060 contains more than 20 each of NRPS and T1PKS BGCs, while the other two strains showing high genomic similarity to *K. albida* NRRL B-24060 only harbor 3 or 4 each of NRPS and T1PKS BGCs. This may be because some of the larger clusters were split into smaller clusters because of poor genome quality, resulting in an inflated number of BGCs for NRRL B-24060. Notably, the exact numbers of BGCs in these *Kutzneria* strains need to be updated when the complete genome sequences are available.

Because *K. albida* DSM 43870 and *K. albida* NRRL-B-24060 are almost identical strains with the former having better genome quality, the diversity and novelty of all the 284 putative BGCs from six high-quality *Kutzneria* genomes were investigated using BiG-SCAPE (Figure 4). Among the 1923 reference BGCs in the MIBiG database, 375 of them were detected in the gene cluster network and all 659 BGCs were organized into 276 gene cluster families (GCFs, cutoff value of 0.6), including 70 singlets (Figure 4). For example, three BGCs from *Kutzneria* strains and three known BGCs from the MIBiG database were grouped into the same GCF with the *epm* BGC, highlighting the promising potential to discover novel macrolides from this genus. Interestingly, only six homologous BGCs were found in all six strains of *Kutzneria*, implying that these BGCs are relatively conserved and may represent a set of ancestral secondary metabolite gene clusters. In addition, many BGCs were solely observed in *K. viridogriseum* DSM 43850 and *K. albida* DSM 43870, which was consistent with their high genomic similarity to each other. For example, one BGC in *K. viridogriseum* DSM 43850 showed high homology with the *hui* BGC from *K. albida* DSM 43870. It is worth noting that many strain-specific BGCs showing high similarity to known BGCs were identified from the network, which deserve further validation. For example, two BGCs homologous to that encoding cinnamycin and caldoniamides A−G, respectively, were identified from *K.* sp. CA-103260. Overall, among the 276 GCFs obtained, only 63 of them contain characterized or known BGCs, suggesting that 77% of the secondary metabolic potential of *Kutzneria* is unexplored.

The detailed secondary metabolic potential of *Kutzneria* was further explored by investigating the diversity and distribution of the key metabolic genes in the predicted secondary metabolite gene clusters. Only gene clusters containing genes encoding cytochrome P450 and identified metabolic enzymes were selected for in-depth analysis and are presented in Figure 5. Cytochrome P450s are a superfamily of enzymes that function as monooxygenases and perform diverse catalytic roles in secondary metabolism.[31] As shown in Figure 5, genes encoding cytochrome P450s from *Kutzneria* strains were found to be distributed in more than 29 clusters, demonstrating the highest diversity of cytochrome P450s among the enzymes analyzed. Moreover, many strain-specific cytochrome P450s were identified based on the network, which suggests promising potential and deserves future investigation.

No homologous BGCs of the *Ktz* BGC were found in the genomes of these *Kutzneria* strains (Figure 4), which could be explained by the existence of the singlets representing KtzA−C, KtzN, and KtzT. Two homologous protein-coding genes of KtzG (adenylation domain) involved in the biosynthesis of kutzneride 2 were identified from the genomes of two *Kutzneria* strains. Interestingly, a total of eight potential flavin-dependent *N*-hydroxylases (including KtzI) were identified from the six genomes of *Kutzneria*, and six of them were located in NRPS or PKS/NRPS BGCs, suggesting their potential to produce L-N^5-hydroxyornithine containing nonribosomal peptides. The distributional differences of these metabolic enzymes in strains of the same genus suggest that these functional genes may evolve at different rates. Besides, homologous proteins of KaPH1 and KalbTG were also identified from *K. viridogriseum* DSM 43850. These findings provide valuable information for the targeted discovery of novel metabolic enzymes from *Kutzneria*.

Seven polyketide cyclases distributed in three distinct clusters were discovered from the putative secondary metabolic enzymes, but BGCs containing six of these polyketide cyclases were mainly presented in different BGC clusters (Figure S1), suggesting that the *Kutzneria* strains are likely to encode many structurally diverse ring-containing polyketide natural products. Previous studies have discovered and characterized four halogenases (KtzD, KtzQ, KtzR, and KthP) from *Kutzneria* sp. 744 (Figure S2), and the metabolic gene network revealed that nine protein-coding genes from the other five *Kutzneria* strains showed high sequence similarity to these halogenase-encoding genes. Notably, eight of these putative halogenase-encoding genes were located in predicted BGCs (mainly NRPS BGCs) (Figure S2), suggesting that these *Kutzneria* strains have the ability to synthesize novel chlorine-containing peptides.

CONCLUSIONS AND FUTURE PERSPECTIVES

To date, only four types of secondary metabolites (macrolide glycosides, cyclic hexadepsipeptides, pyrrolopyrimidine, and modified shikimate) have been isolated and characterized from *Kutzneria*, and the core metabolic enzymes involved in the biosynthesis of epemicins A and B, huimycin, chloramphenicol, and kutzneride 2 have been experimentally validated, especially for kutzneride 2. The sources, functions, and coding genes for all characterized enzymes or proteins from *Kutzneria* strains are also summarized in this review.

In-depth comparative genomic analysis indicates that six high-quality genomes of *Kutzneria* strains encode 284 BGCs that are organized into 276 GCFs, and only 63 GCFs contain characterized or known BGCs, suggesting that secondary metabolites encoded by BGCs in the other 213 GCFs are unknown and most likely represent novel natural products. In the future, the secondary metabolites encoded by these cryptic BGCs can be obtained by means of heterologous expression, OSMAC, and HiTES.[32−34]

In addition, plenty of homologous proteins of key metabolic enzymes have been identified from the genomes of *Kutzneria* strains, including cytochrome P450s, halogenases, and flavin-dependent *N*-hydroxylases. Some of these enzymes show some

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127

degree of species-specificity. Future studies may explore the structure−activity relationships of these homologous enzymes through heterologous expression, which will also lay an important foundation for the accurate prediction of natural product structure from genomic data.

EXPERIMENTAL SECTION

Genome Sequences. All genomes of *Kutzneria* strains were retrieved from the NCBI GenBank database. The NCBI accession number, genome size, GC content, and gene numbers of all the genomes were obtained and summarized in Table S1. Genome integrity was assessed by CheckM version 1.0.12.[35]

Secondary Metabolite Gene Cluster Prediction and Analysis. Gene cluster prediction and networking were performed according to our recent report.[36] All genomes were processed using antiSMASH 6.0 with the default parameters.[37] The detailed information about the protein-coding genes in putative gene clusters is summarized in Table S4. Only BGCs from high-quality genomes were collected to construct the similarity network using biosynthetic genes similarity clustering and prospecting engine (BiG-SCAPE).[38] BGCs were clustered into GCFs at a 0.6 cutoff.

Identification of the Homologous Proteins of the Characterized Metabolic Enzymes from *Kutzneria*. All protein-coding genes annotated in the six high-quality *Kutzneria* genomes were extracted from the GBK files generated by antiSMASH and then submitted to the EFI−Enzyme Similarity Tool (EFI−EST) to identify the homologous proteins of previously characterized metabolic enzymes by constructing a similarity network of secondary metabolic genes.[39]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.3c00007.

Figures of the distribution of putative polyketide cyclases, halogenases, and flavin-dependent *N*-hydroxylases in *Kutzneria* BGCs; comparative analysis of BGCs containing putative halogenase-encoding genes in *Kutzneria* strains (PDF)

Tables of genomic features and BGC composition in *Kutzneria* genomes; information about the BGCs presented in Figure 4; information about the protein-coding genes presented in Figure 5; information about the protein-coding genes in all *Kutzneria* BGCs (ZIP)

AUTHOR INFORMATION

Corresponding Authors

Hong Wang − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China;* orcid.org/0000-0003-0058-060X; Phone: 86-571-8832-0622; Email: hongw@zjut.edu.cn

Xue-Wei Xu − *Key Laboratory of Marine Ecosystem and Biogeochemistry, Ministry of Natural Resources & Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China*; Phone: 86-571-8832-0622; Email: xuxw@sio.org.cn

Authors

Bin Wei − *Key Laboratory of Marine Ecosystem and Biogeochemistry, Ministry of Natural Resources & Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China; College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China;* orcid.org/0000-0001-7362-1228

Ao-Qi Du − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Ti-Ti Ying − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Gang-Ao Hu − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Zhen-Yi Zhou − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China;* orcid.org/0000-0003-1342-6054

Wen-Chao Yu − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Jing He − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Yan-Lei Yu − *College of Pharmaceutical Science & Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Zhejiang University of Technology, Hangzhou 310014, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.3c00007

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (Nos. 2022YFC2804700 and 2022YFC2804104) and the Fundamental Research Funds for the Provincial Universities of Zhejiang (No. RF-A2022013). The authors also thank the support from the Zhejiang Provincial Key Laboratory of TCM for Innovative R & D and Digital Intelligent Manufacturing of TCM Great Health

Products and Zhejiang International Sci-Tech Cooperation Base for the Exploitation and Utilization of Nature Product.

REFERENCES

(1) Demain, A. L. *J. Ind. Microbiol. Biotechnol* **2014**, *41* (2), 185−201.
(2) Wei, B.; Du, A. Q.; Zhou, Z. Y.; Lai, C.; Yu, W. C.; Yu, J. B.; Yu, Y. L.; Chen, J. W.; Zhang, H. W.; Xu, X. W.; Wang, H. *Environ. Microbiol.* **2021**, *23* (11), 6981−6992.
(3) Tiwari, K.; Gupta, R. K. *Crit. Rev. Biotechnol.* **2012**, *32* (2), 108−132.
(4) Kontou, E. E.; Gren, T.; Ortiz-López, F. J.; Thomsen, E.; Oves-Costales, D.; Díaz, C.; de la Cruz, M.; Jiang, X.; Jørgensen, T. S.; Blin, K.; Charusanti, P. *ACS Chem. Biol.* **2021**, *16* (8), 1456−1468.
(5) Takahashi, Y. *J. Antibiot.* **2017**, *70* (5), 506−513.
(6) Yu, T. W.; Bai, L.; Clade, D.; Hoffmann, D.; Toelzer, S.; Trinh, K. Q.; Xu, J.; Moss, S. J.; Leistner, E.; Floss, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (12), 7968−7973.
(7) Stackebrandt, E.; Kroppenstedt, R. M.; Jahnke, K. D.; Kemmerling, C.; Gürtler, H. *Int. J. Syst. Evol. Microbiol.* **1994**, *44* (2), 265−269.
(8) Rebets, Y.; Tokovenko, B.; Lushchyk, I.; Rückert, C.; Zaburannyi, N.; Bechthold, A.; Kalinowski, J.; Luzhetskyy, A. *BMC Genom.* **2014**, *15* (1), 1−15.
(9) Devi, T. S.; Vijay, K.; Vidhyavathi, R. M.; Kumar, P.; Govarthanan, M.; Kavitha, T. *Arch. Microbiol.* **2021**, *203* (7), 4051−4064.
(10) Broberg, A.; Menkis, A.; Vasiliauskas, R. *J. Nat. Prod.* **2006**, *69* (1), 97−102.
(11) Fujimori, D. G.; Hrvatin, S.; Neumann, C. S.; Strieker, M.; Marahiel, M. A.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (42), 16498−16503.
(12) Heemstra Jr, J. R.; Walsh, C. T. *J. Am. Chem. Soc.* **2008**, *130* (43), 14024−14025.
(13) Schmitz, L. M.; Kinner, A.; Althoff, K.; Rosenthal, K.; Lütz, S. *ChemBioChem.* **2021**, *22* (13), 2266−2274.
(14) Okuda, T.; Ito, Y.; Yamaguchi, T.; Furumai, T.; Suzuki, M.; Tsuruoka, M. *J. Antibiot.* **1966**, *19* (2), 85−87.
(15) Harada, K. I.; Kimura, I.; Sakazaki, T.; Murata, H.; Suzuki, M. *J. Antibiot.* **1989**, *42* (7), 1056−1062.
(16) Ikemoto, T.; Katayama, T.; Shiraishi, A.; Haneishi, T. *J. Antibiot.* **1983**, *36* (9), 1097−1100.
(17) Shuai, H.; Myronovskyi, M.; Nadmid, S.; Luzhetskyy, A. *Biomolecules* **2020**, *10* (7), 1074.
(18) Rosenbach, L. M.; Caviles, A. P.; Mitus, W. J. *N. Engl. J. Med.* **1960**, *263* (15), 724−728.
(19) Tamura, A.; Takeda, I.; Naruto, S.; Yoshimura, Y. *J. Antibiot.* **1971**, *24* (4), 270−270.
(20) Pohanka, A.; Menkis, A.; Levenfors, J.; Broberg, A. *J. Nat. Prod.* **2006**, *69* (12), 1776−1781.
(21) Louzoun Zada, S.; Green, K. D.; Shrestha, S. K.; Herzog, I. M.; Garneau-Tsodikova, S.; Fridman, M. *ACS Infect. Dis.* **2018**, *42* (7), 1121−1129.
(22) He, J.; Magarvey, N.; Piraee, M.; Vining, L. C. *Microbiology* **2001**, *147* (10), 2817−2829.
(23) Fernández-Martínez, L. T.; Borsetto, C.; Gomez-Escribano, J. P.; Bibb, M. J.; Al-Bassam, M. M.; Chandra, G.; Bibb, M. J. *Antimicrob. Agents Chemother.* **2014**, *58* (12), 7441−7450.
(24) Neumann, C. S.; Walsh, C. T. *J. Am. Chem. Soc.* **2008**, *130* (43), 14022−14023.
(25) Zolova, O. E.; Garneau-Tsodikova, S. *J. Antibiot.* **2014**, *67* (1), 59−64.
(26) Du, Y. L.; He, H. Y.; Higgins, M. A.; Ryan, K. S. *Nat. Chem. Biol.* **2017**, *13* (8), 836−838.
(27) Setser, J. W.; Heemstra, J. R., Jr; Walsh, C. T.; Drennan, C. L. *Biochemistry* **2014**, *53* (38), 6063−6077.
(28) Jiang, W.; Heemstra, J. R., Jr; Forseth, R. R.; Neumann, C. S.; Manaviazar, S.; Schroeder, F. C.; Hale, K. J.; Walsh, C. T. *Biochemistry* **2011**, *50* (27), 6063−6072.
(29) Jing, X.; Wang, X.; Zhang, W.; An, J.; Luo, P.; Nie, Y.; Xu, Y. *ACS Omega* **2019**, *4* (5), 8350−8358.
(30) Steffen, W.; Ko, F. C.; Patel, J.; Lyamichev, V.; Albert, T. J.; Benz, J.; Rudolph, M. G.; Bergmann, F.; Streidl, T.; Kratzsch, P.; Boenitz-Dulat, M. *J. Biol. Chem.* **2017**, *292* (38), 15622−15635.
(31) Greule, A.; Stok, J. E.; De Voss, J. J.; Cryle, M. J. *Nat. Prod. Rep.* **2018**, *35* (8), 757−791.
(32) Wang, H.; Li, Z.; Jia, R.; Hou, Y.; Yin, J.; Bian, X.; Li, A.; Müller, R.; Stewart, A. F.; Fu, J.; Zhang, Y. *Nat. Protoc.* **2016**, *11* (7), 1175−1190.
(33) Pan, R.; Bai, X.; Chen, J.; Zhang, H.; Wang, H. *Front. Microbiol.* **2019**, *10*, 294.
(34) Xu, F.; Wu, Y.; Zhang, C.; Davis, K. M.; Moon, K.; Bushin, L. B.; Seyedsayamdost, M. R. *Nat. Chem. Biol.* **2019**, *15* (2), 161−168.
(35) Parks, D. H.; Imelfort, M.; Skennerton, C. T.; Hugenholtz, P.; Tyson, G. W. *Genome Res.* **2015**, *25*, 1043−1055.
(36) Du, A. Q.; Ying, T. T.; Zhou, Z. Y.; Yu, W. C.; Hu, G. A.; Luo, X.; Ma, M. J.; Yu, Y. L.; Wang, H.; Wei, B. *Environ. Microbiol. Rep.* **2022**, *14* (6), 917−925.
(37) Blin, K.; Shaw, S.; Kloosterman, A. M.; Charlop-Powers, Z.; Van Wezel, G. P.; Medema, M. H.; Weber, T. *Nucleic Acids Res.* **2021**, *49* (W1), W29−W35.
(38) Navarro-Muñoz, J. C.; Selem-Mojica, N.; Mullowney, M. W.; Kautsar, S. A.; Tryon, J. H.; Parkinson, E. I.; De Los Santos, E. L.; Yeong, M.; Cruz-Morales, P.; Abubucker, S.; Roeters, A.; et al. *Nat. Chem. Biol.* **2020**, *16* (1), 60−68.
(39) Zallot, R.; Oberg, N.; Gerlt, J. A. *Biochemistry* **2019**, *41*, 4169−4182.

https://doi.org/10.1021/acs.jnatprod.3c00007
J. Nat. Prod. 2023, 86, 1120−1127