

Electronic Supplementary Materials

The impact of heterologous regulatory genes from lipodepsipeptide biosynthetic gene clusters on the production of teicoplanin and A40926

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Table S1. Information of StrR-like PSRs coded within LDP BGCs (A), as well as in antibiotic BGCs available in MIBiG (B).

MIBiG/GenBank accession number for the hit	Protein ID	E- value	Amino acid sequence identity	Microorganism	Antibiotic	Class	Reference
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	Ramo5			<i>Actinoplanes ramoplaninifer</i> ATCC 33076	Ramoplanin	LDP	[1]
(A) StrR-like pathway specific regulators from LDP BGCs							
BGC0000341	End22 ABD65942	4.53e- 58	49.2%	<i>Streptomyces fungicidicus</i> ATCC 21013	Enduracidin	LDP	[2]
BGC0000341	End24 ABD65944	2.28e- 71	43.2%	<i>Streptomyces fungicidicus</i> ATCC 21013	Enduracidin	LDP	[2]
NZ_FMIB0100000 2	Chers28 WP_09130 5478	8.99e- 128	71.6%	<i>Micromonospora chersina</i> DSM 44151	Chersina-mycin	LDP	[3]
NZ_KB913037	Not annotated	2.25e- 71	47.2%	<i>Amycolatopsis balhimycina</i> FH 1894	Putative LDP	LDP	[3]
NZ_KB913037	Not annotated	9.46e- 69	45.8%	<i>Amycolatopsis balhimycina</i> FH 1894	Putative LDP	LDP	[3]
CP016174	ANN21820	1.97e- 71	45.8%	<i>Amycolatopsis orientalis</i> B-37	Putative LDP	LDP	[3]
CP016174	ANN21819	5.83e- 69	45.2%	<i>Amycolatopsis orientalis</i> B-37	Putative LDP	LDP	[3]
NZ ASJB01000042	WP_23578 3490	4.77e- 72	46.0%	<i>Amycolatopsis orientalis</i> DSM 40040/KCTC 9412	Putative LDP	LDP	[3]
NZ ASJB01000042	WP_23578 3491	1.02e- 69	45.8%	<i>Amycolatopsis orientalis</i> DSM 40040/KCTC 9412	Putative LDP	LDP	[3]
LT629775	SDT43639. 1	3.26e- 56	41.1%	<i>Streptomyces</i> sp. TLI_053	Putative LDP	LDP	[3]
VFOE01000001	TQL19429	1.78e- 72	49.2%	<i>Streptomyces</i> sp. SLBN-134 Ga0314649_11	Putative LDP	LDP	[3]
VFOE01000001	TQL19431	3.02e- 63	43.1%	<i>Streptomyces</i> sp. SLBN-134 Ga0314649_11	Putative LDP	LDP	[3]
(B) StrR-like pathway specific regulators of BCGs from MIBiG database							
BGC0000021	ApoR3 AEP40926	1.53e- 70	48.2%	<i>Nocardiopsis</i> sp. FU 40	Apoptolidin	Type I polyketide	[4]
BGC0001381	NbrR10 AJO72769	7.30e- 49	39.3%	<i>Nocardia terpenica</i> IFM 0406	Brasilinolide	Type I polyketide	[5]
BGC0000159	TtmQ ABW96549	4.85e- 53	41.3%	<i>Streptomyces spiroverticillatus</i> CGMCC:4.1749	Tautomycin	Modular type I polyketide	[6]

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
BGC0000112	AAM78007	2.14e-58	40.2%	<i>Streptomyces carzinostaticus</i> ssp. <i>neocarzinostaticus</i> ATCC 15944	Neocarzinostatin	Enediynes, type I polyketide	[7]
BGC0000965	AAL06695	2.44e-65	42.2%	<i>Streptomyces globisporus</i> C-1027	C-1027	Enediynes, type I polyketide	[8]
BGC0001008	MdpR1 ABY65997	1.20e-64	46.6%	<i>Actinomadura madurae</i> ATCC 39144	Maduropeptin	Enediynes, type I polyketide	[9]
BGC0001397	ALU98457	2.44e-65	42.2%	<i>Streptomyces globisporus</i> C-1027	C-1027	Enediynes, type I polyketide	[10]
BGC0001584	ANY94466	1.79e-64	42.5%	<i>Streptomyces</i> sp. CB02366	C-1027	Enediynes, type I polyketide	[11]
BGC0001904	ALJ99870	6.16e-67	44.2%	<i>Micromonospora rosaria</i> SCSIO N160	Fluostatin	Type II polyketide	[12]
BGC0000240	Lom15 AHZ61849	6.50e-53	37.8%	<i>Salinispora pacifica</i> DPJ-0016	Lomaiviticin	Type II polyketide	[13]
BGC0000241	ABP54638	9.22e-54	36.8%	<i>Salinispora tropica</i> CNB-440	Lomaiviticin	Type II polyketide	[13]
BGC0000267	SaqI ACP19349	1.68e-76	49.7%	<i>Micromonospora</i> sp. Tü 6368	Saquayamycin	Type II polyketide	[14]
BGC0001596	WP_15939 3028	9.99e-61	45.1%	<i>Streptomyces albus</i> DSM 41398	Fluostatins	Type II polyketide	[15]
BGC0001693	ARD70863	2.18e-58	40.9%	<i>Micromonospora echinospora</i> SCSIO 04089	Nenestatin	Type II polyketide	[16]
BGC0000690	StrR CAA07385	1.11e-72	46.5%	<i>Streptomyces glaucescens</i> GLA.0	Hydroxystreptomycin	Aminoglycoside	[17]
BGC0000698	ABC42540	9.85e-58	42.8%	<i>Streptomyces hygroscopicus</i> NRRL 2388	Hygromycin	Aminoglycoside	[18]
BGC0000702	BAD20753	1.18e-63	44.9%	<i>Streptomyces kanamyceticus</i> ATCC 12853	Kanamycin	Aminoglycoside	[19]
BGC0000716	SpcR AAB66654	2.15e-66	43.6%	<i>Streptomyces netropsis</i> JCM 4063	Spectinomycin	Aminoglycoside	[20]
BGC0000724	StrR BAG22760	3.37e-70	47.2%	<i>Streptomyces griseus</i> ssp. <i>griseus</i> NBRC 13350	Streptomycin	Aminoglycoside	[21]
BGC0000832	CloG AAN65222	1.92e-59	43.9%	<i>Streptomyces roseochromogenus</i> subsp. <i>oscitans</i> DS 12.976	Clorobiocin	Aminocoumarin	[22]
BGC0000833	CouG AAG29778	2.35e-56	41.3%	<i>Streptomyces rishiriensis</i> DSM 40489	Coumermycin	Aminocoumarin	[23]
BGC0000834	NovG AAF67500	4.28e-53	41.8%	<i>Streptomyces niveus</i> NCIMB 9219	Novobiocin	Aminocoumarin	[24]

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
BGC0001783	StrT2 AFW04588	2.79e- 61	43.4%	<i>Streptomyces flocculus</i> CGMCC 4.1223	Streptonigrin	Aminoquinon e alkaloid	[25]
BGC0000935	EsmT1 AFB35624	3.61e- 62	43.4%	<i>Streptomyces antibioticus</i> Tü 2706	Esmeraldin	Phenazine	[26]
BGC0000679	EWM63064	1.08e- 70	48.1%	<i>Micromonospora sp. M42</i>	Diazepinomicin	Terpene	[27]
BGC0002591	ARM20266	1.80e- 59	46.5%	<i>Streptomyces</i> sp. NA04227	Aurachin	ladderane,terp ene	[28]
BGC0001387	AMP46602	2.21e- 60	41.7%	<i>Streptomyces calvus</i> ATCC 13382	Nucleocidin	Fluorinated ribonucleoside	[29]
BGC0001985	QER91000	6.25e- 46	36.5%	<i>Streptomyces candidus</i> NRRL 3601	Pyrazofurin	C-nucleoside	[30]
BGC0002039	FocG AVW82900	1.99e- 48	37.7%	<i>Nocardia interforma</i> ATCC 21072	Coformycin, formycin	C-nucleoside	[31]
BGC0001193	DtpR2 AJI44174	9.70e- 76	48.6%	<i>Saccharothrix algeriensis</i> NRRL B-24137	Thiolutin	Dithiopyrrol one	[32]
BGC0000893	CCA54200	7.10e- 58	43.5%	<i>Streptomyces venezuelae</i> ATCC 10712	Chloramphenico l	NRP	[33]
BGC0001620	ASX95224	6.85e- 70	47.9%	<i>Streptomyces atratus</i> SCSIO_ZH16	Ilamycin	Cyclic NRP	[34]
BGC0001763	RufA BBA20948	5.12e- 70	47.9%	<i>Streptomyces atratus</i> SCSIO_ZH16	Rufomycin	Cyclic NRP	[35]
BGC0001233	FegB ALK27899	1.03e- 66	43.5%	<i>Streptomyces</i> sp. DSM 11171	Feglymycin	Linear NRP	[36]
BGC0000311	Bbr CAG25754	6.57e- 71	45.9%	<i>Amycolatopsis balhimycina</i> DSM 5908	Balhimycin	Type I GPA	[37]
BGC0000455	VtrR AEI58862	5.70e- 74	47.2%	<i>Amycolatopsis orientalis</i> HCCB10007	Vancomycin	Type I GPA	[38]
BGC0001459	OKA09420	3.95e- 71	45.6%	<i>Amycolatopsis regifaucium</i> GY080	Decaplanin	Type I GPA	[39]
BGC0001460	EME52993	1.28e- 71	45.9%	<i>Amycolatopsis decaplanina</i> DSM 44594	Decaplanin	Type I GPA	[40]
BGC0001461	OLZ50883	5.07e- 70	46.6%	<i>Amycolatopsis keratiniphila</i> ssp. <i>nogabecina</i> FH 1893	Nogabecin	Type II GPA	[41]
BGC0001462	OLZ52648	5.65e- 71	47.0%	<i>Amycolatopsis coloradensis</i> DSM 44225	Avoparcin	Type II GPA	[42]
BGC0001955	AYA22339	4.13e- 70	46.6%	<i>Amycolatopsis keratiniphila</i> NRRL B24117	Keratinimicin	Type II GPA	[43]

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
BGC0001178	AGS77330	8.30e-69	46.3%	<i>Actinoplanes</i> sp. ATCC 53533	UK-68,597	Type III GPA	[44]
BGC0000290	StaQ AAM80553	6.10e-67	43.7%	<i>Streptomyces toyocaensis</i> NRRL 15009	A47934	Type III GPA	[45]
BGC0000418	AIE77054	9.25e-73	47.2%	<i>Amycolatopsis lurida</i> NRRL 2430	Ristocetin	Type III GPA	[46]
BGC0000419	AIG79246	4.05e-72	46.9%	<i>Amycolatopsis japonica</i> MG417-CF17	Ristocetin	Type III GPA	[47]
BGC0000440	Tei15* CAE53369	5.08e-68	44.5%	<i>Actinoplanes teichomyceticus</i> ATCC 31121	Teicoplanin	Type IV GPA	[48]
BGC0002344	NocRII QYC40303	1.87e-72	45.9%	<i>Nonomuraea coxensis</i> DSM 45129	A50926	Type IV GPA	[49]
BGC0000289	Dbv4 CAD91199	2.35e-73	46.5%	<i>Nonomuraea gerenzanensis</i> ATCC 39727	A40926	Type IV GPA	[50]
BGC0002637	RSO11558	2.69e-78	48.4%	<i>Streptomyces</i> sp. WAC 06783	Rimomycin	Type V GPA	[51]
BGC0002638	MCC50367 81	2.31e-78	47.7%	<i>Streptomyces</i> sp. WAC 00631	Misaugamycin	Type V GPA	[51]
BGC0001635	AQZ71349	1.28e-64	47.1%	<i>Nonomuraea</i> sp. ATCC 55076	Kistamicin	Type V GPA	[52]
BGC0000326	AAK81822	3.76e-68	45.5%	<i>Streptomyces lavendulae</i> SANK 60477	Complestatin	Type V GPA	[53]

Table S2. Summary of the StrR-like pathway specific regulators of some GPA BGCs absent in MIBiG.

GenBank accession number for the hit	Protein ID	E value	AA sequence identity	Organism	Antibiotic	Class	Reference
HM486074	ADU56075	2.59e-92	57.3%	Uncultured organism CA37	Putative GPA (predicted <i>in silico</i>)	GPA	[54]
HM486074	ADU56074	2.25e-89	54.5%	Uncultured organism CA37	Putative GPA (predicted <i>in silico</i>)	GPA	[54]
HM486074	ADU56064	3.41e-66	45.8%	Uncultured organism CA37	Putative GPA (predicted <i>in silico</i>)	GPA	[54]
HM486075	ADU56085	1.38e-72	45.9%	Uncultured organism CA878	Putative GPA (predicted <i>in silico</i>)	GPA	[54]
HM486076	ADU56159	4.61e-70	45.4%	Uncultured organism CA915	Putative GPA (predicted <i>in silico</i>)	GPA	[54]
KF264554	AGS49770	2.70e-67	45.2%	Uncultured bacterium esnapd15	Putative GPA (predicted <i>in silico</i>)	GPA	[55]
EU874253	ACJ60984	6.16e-74	46.5%	Uncultured soil bacterium clone D30	Putative GPA (predicted <i>in silico</i>)	GPA	[56]
EU874252	ACJ60943	8.97e-73	47.1%	Uncultured soil bacterium clone B128	Putative GPA (predicted <i>in silico</i>)	GPA	[56]
JX026280	AGF91741	9.20e-73	46.8%	<i>Streptomyces</i> sp. WAC1420	Pekiskomycin	Type I GPA	[57]
CP016174	ANN20081	3.93e-72	45.9%	<i>Amycolatopsis orientalis</i> B-37	Norvancomycin	Type I GPA	[58]
QHJI01000006	RSN30133	6.92e-75	47.2%	<i>Amycolatopsis</i> sp. WAC 04169	Dimethylvancomycin	Type I GPA	[59]
QHKI01000005	RSM88020	8.46e-74	47.9%	<i>Kibdelosporangium aridum</i> A82846	Chloroeremomycin	Type I GPA	[59]
JPLW01000007	KFZ77397	7.28e-73	46.9%	<i>Amycolatopsis</i> sp. MJM2582	Ristocetin	Type III GPA	[46]
CP024972	QXV59348	4.49e-69	46.5%	<i>Amycolatopsis</i> sp. TNS106	Ristocetin	Type III GPA	[60]
MUXN01000025	OOC02329	1.48e-72	46.5%	<i>Amycolatopsis azurea</i> DSM 43854	Azureomycin	Type III GPA	[61]
QHGX01000008	RSN28334	3.13e-73	47.2%	<i>Amycolatopsis</i> sp. WAC 01416	GP01416	Type IV GPA	[59]
CP029618	AZM51136	2.56e-75	49.2%	<i>Streptomyces</i> sp. WAC 06738	GP6738	Type IV GPA	[59]
JMGY01000008	KEF19256	2.32e-78	48.4%	<i>Streptomyces rimosus</i> R6-500MV9-R8	Rimomycin	Type V GPA	[51]
MCNU01000022	OFA57931	2.10e-74	46.4%	<i>Streptomyces fradiae</i> Olg4R	Miasugamycin	Type V GPA	[51]

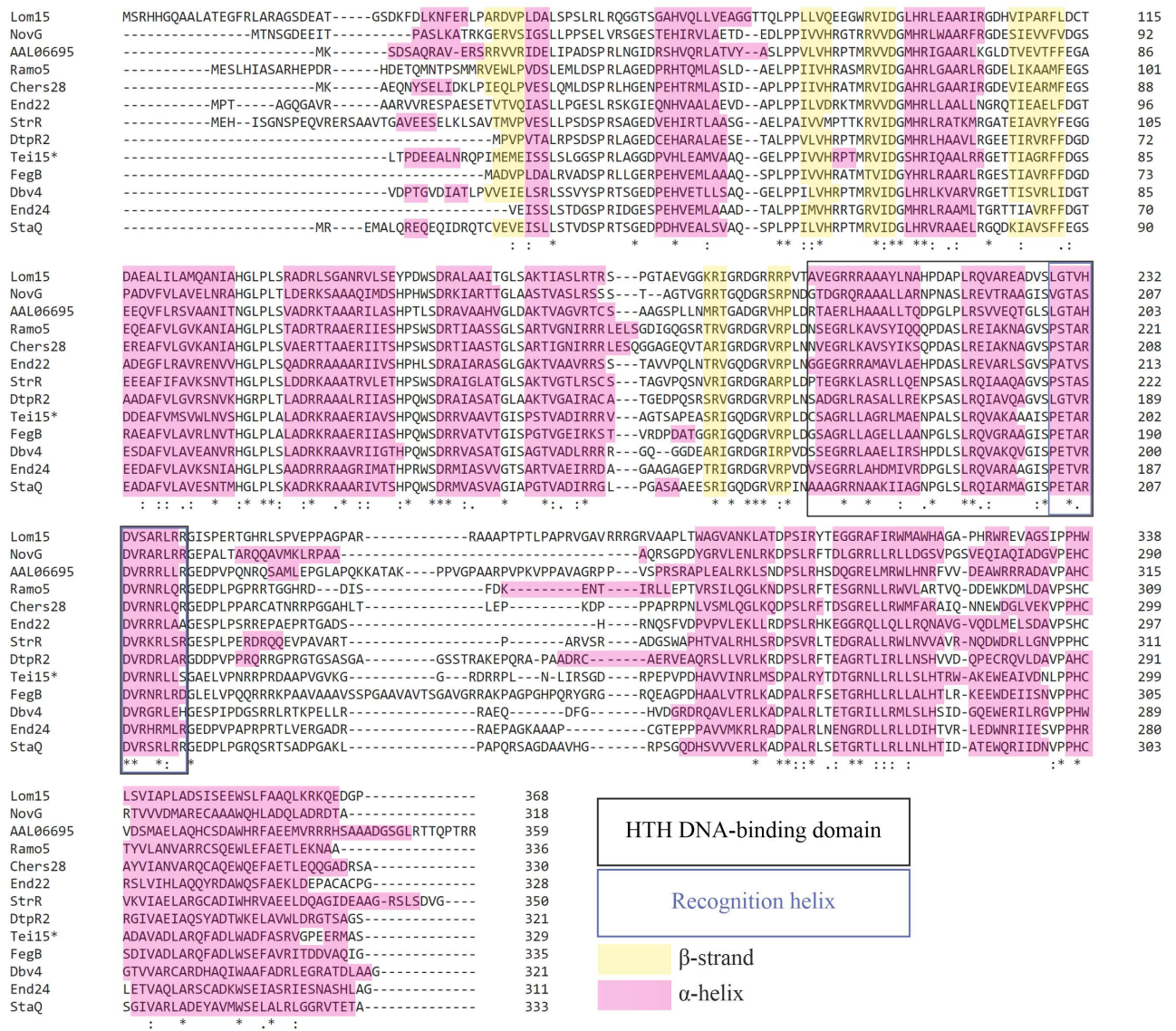


Figure S2. Multiple amino acid sequence alignment of 13 StrR-like PSRs that represent main clades of the phylogenetic tree from Figure S1, where the secondary structure of the proteins is shown. Secondary structure modelling was performed in CHIMERA X using AlphaFold [63–65]. Multiple amino acid sequence alignment was done using Clustal Omega [66].

Supplementary References

1. Farnet, C.M. GENE CLUSTER FOR RAMOPLANIN BIOSYNTHESIS 2005, 222.
2. Yin, X.; Zabriskie, T.M. The enduracidin biosynthetic gene cluster from *Streptomyces fungicidicus*. *Microbiology* **2006**, 152, 2969–2983, doi:10.1099/mic.0.29043-0.
3. Morgan, K.T.; Zheng, J.; McCafferty, D.G. Discovery of Six Ramoplanin Family Gene Clusters and the Lipoglycopeptide Chersinamycin**. *ChemBioChem* **2021**, 22, 176–185, doi:10.1002/cbic.202000555.
4. Du, Y.; Derewacz, D.K.; Deguire, S.M.; Teske, J.; Ravel, J.; Sulikowski, G.A.; Bachmann, B.O. Biosynthesis of the apoptolidins in *Nocardioopsis* sp. FU 40. *Tetrahedron* **2011**, 67, 6568–6575, doi:10.1016/J.TET.2011.05.106.
5. Chiu, H.T.; Weng, C.P.; Lin, Y.C.; Chen, K.H. Target-specific identification and characterization of the putative gene cluster for brasilinolide biosynthesis revealing the mechanistic insights and combinatorial synthetic utility of 2-deoxy-l-fucose biosynthetic enzymes. *Org. Biomol. Chem.* **2016**, 14, 1988–2006, doi:10.1039/C5OB02292D.
6. Li, W.; Ju, J.; Rajske, S.R.; Osada, H.; Shen, B. Characterization of the tautomycin biosynthetic gene cluster from *Streptomyces spiroverticillatus* unveiling new insights into dialkylmaleic anhydride and polyketide biosynthesis. *J. Biol. Chem.* **2008**, 283, 28607–28617, doi:10.1074/JBC.M804279200.
7. Liu, W.; Nonaka, K.; Nie, L.; Zhang, J.; Christenson, S.D.; Bae, J.; Van Lanen, S.G.; Zazopoulos, E.; Farnet, C.M.; Yang, C.F.; et al. The neocarzinostatin biosynthetic gene cluster from *Streptomyces carzinostaticus* ATCC 15944 involving two iterative type I polyketide synthases. *Chem. Biol.* **2005**, 12, 293–302, doi:10.1016/J.CHEMBIOL.2004.12.013.
8. Liu, W.; Christenson, S.D.; Standage, S.; Shen, B. Biosynthesis of the enediyne antitumor antibiotic C-1027. *Science* (80-.). **2002**, 297, 1170–1173, doi:10.1126/science.1072110.
9. Van Lanen, S.G.; Oh, T.J.; Liu, W.; Wendt-Pienkowski, E.; Shen, B. Characterization of the maduropeptin biosynthetic gene cluster from *Actinomadura madurae* ATCC 39144 supporting a unifying paradigm for enediyne biosynthesis. *J. Am. Chem. Soc.* **2007**, 129, 13082–13094, doi:10.1021/JA073275O.
10. Li, X.; Lei, X.; Zhang, C.; Jiang, Z.; Shi, Y.; Wang, S.; Wang, L.; Hong, B. Complete genome sequence of *Streptomyces globisporus* C-1027, the producer of an enediyne antibiotic lidamycin. *J. Biotechnol.* **2016**, 222, 9–10, doi:10.1016/j.jbiotec.2016.02.004.
11. Yan, X.; Ge, H.; Huang, T.; Hindra; Yang, D.; Teng, Q.; Crnovčić, I.; Li, X.; Rudolf, J.D.; Lohman, J.R.; et al. Strain prioritization and genome mining for enediyne natural products. *MBio* **2016**, 7, doi:10.1128/mBio.02104-16.

12. Yang, C.; Huang, C.; Zhang, W.; Zhu, Y.; Zhang, C. Heterologous Expression of Fluostatin Gene Cluster Leads to a Bioactive Heterodimer. *Org. Lett.* **2015**, *17*, 5324–5327, doi:10.1021/ACS.ORGLETT.5B02683.
13. Janso, J.E.; Haltli, B.A.; Eustáquio, A.S.; Kulowski, K.; Waldman, A.J.; Zha, L.; Nakamura, H.; Bernan, V.S.; He, H.; Carter, G.T.; et al. Discovery of the lomaiviticin biosynthetic gene cluster in *Salinispora pacifica*. *Tetrahedron* **2014**, *70*, 4156–4164, doi:10.1016/j.tet.2014.03.009.
14. Erb, A.; Luzhetskyy, A.; Hardter, U.; Bechthold, A. Cloning and sequencing of the biosynthetic gene cluster for saquayamycin Z and galtamycin B and the elucidation of the assembly of their saccharide chains. *ChemBiochem* **2009**, *10*, 1392–1401, doi:10.1002/CBIC.200900054.
15. Jin, J.; Yang, X.; Liu, T.; Xiao, H.; Wang, G.; Zhou, M.; Liu, F.; Zhang, Y.; Liu, D.; Chen, M.; et al. Fluostatins M-Q Featuring a 6-5-6-6 Ring Skeleton and High Oxidized A-Rings from Marine *Streptomyces* sp. PKU-MA00045. *Mar. Drugs* **2018**, *16*, doi:10.3390/MD16030087.
16. Jiang, X.; Zhang, Q.; Zhu, Y.; Nie, F.; Wu, Z.; Yang, C.; Zhang, L.; Tian, X.; Zhang, C. Isolation, structure elucidation and biosynthesis of benzo[b]fluorene nenestatin A from deep-sea derived *Micromonospora echinospora* SCSIO 04089. *Tetrahedron* **2017**, *73*, 3585–3590, doi:10.1016/J.TET.2017.03.054.
17. Beyer, S.; Distler, J.; Piepersberg, W. The str gene cluster for the biosynthesis of 5'-hydroxystreptomycin in *Streptomyces glaucescens* GLA.0 (ETH 22794): New operons and evidence for pathway-specific regulation by StrR. *Mol. Gen. Genet.* **1996**, *250*, 775–784, doi:10.1007/BF02172990.
18. Palaniappan, N.; Ayers, S.; Gupta, S.; Habib, E.S.; Reynolds, K.A. Production of hygromycin A analogs in *Streptomyces hygrosopicus* NRRL 2388 through identification and manipulation of the biosynthetic gene cluster. *Chem. Biol.* **2006**, *13*, 753–764, doi:10.1016/J.CHEMBIOL.2006.05.013.
19. Yanai, K.; Murakami, T. The kanamycin biosynthetic gene cluster from *Streptomyces kanamyceticus*. *J. Antibiot. (Tokyo)*. **2004**, *57*, 351–354, doi:10.7164/ANTIBIOTICS.57.351.
20. Lyutskanova, D.; Distler, J.; Altenbuchner, J. A spectinomycin resistance determinant from the spectinomycin producer *Streptomyces flavopersicus*. *Microbiology* **1997**, *143* (Pt 7), 2135–2143, doi:10.1099/00221287-143-7-2135.
21. Ohnishi, Y.; Ishikawa, J.; Hara, H.; Suzuki, H.; Ikenoya, M.; Ikeda, H.; Yamashita, A.; Hattori, M.; Horinouchi, S. Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. *J. Bacteriol.* **2008**, *190*, 4050–4060, doi:10.1128/JB.00204-08.
22. Pojer, F.; Li, S.M.; Heide, L. Molecular cloning and sequence analysis of the clorobiocin biosynthetic gene cluster: New insights into the biosynthesis of aminocoumarin

antibiotics. *Microbiology* **2002**, *148*, 3901–3911, doi:10.1099/00221287-148-12-3901.

23. Wang, Z.X.; Li, S.M.; Heide, L. Identification of the coumermycin A1 biosynthetic gene cluster of streptomyces rishiriensis DSM 40489. *Antimicrob. Agents Chemother.* **2000**, *44*, 3040–3048, doi:10.1128/AAC.44.11.3040-3048.2000.
24. Steffensky, M.; Mühlenweg, A.; Wang, Z.X.; Li, S.M.; Heide, L. Identification of the novobiocin biosynthetic gene cluster of Streptomyces spheroides NCIB 11891. *Antimicrob. Agents Chemother.* **2000**, *44*, 1214–1222, doi:10.1128/AAC.44.5.1214-1222.2000.
25. Xu, F.; Kong, D.; He, X.; Zhang, Z.; Han, M.; Xie, X.; Wang, P.; Cheng, H.; Tao, M.; Zhang, L.; et al. Characterization of streptonigrin biosynthesis reveals a cryptic carboxyl methylation and an unusual oxidative cleavage of a N-C bond. *J. Am. Chem. Soc.* **2013**, *135*, 1739–1748, doi:10.1021/JA3069243.
26. Rui, Z.; Ye, M.; Wang, S.; Fujikawa, K.; Akerele, B.; Aung, M.; Floss, H.G.; Zhang, W.; Yu, T.W. Insights into a divergent phenazine biosynthetic pathway governed by a plasmid-born esmeraldin gene cluster. *Chem. Biol.* **2012**, *19*, 1116–1125, doi:10.1016/J.CHEMBIOL.2012.07.025.
27. McAlpine, J.B.; Banskota, A.H.; Charan, R.D.; Schlingmann, G.; Zazopoulos, E.; Pirae, M.; Janso, J.; Bernan, V.S.; Aouidate, M.; Farnet, C.M.; et al. Biosynthesis of diazepinomicin/ECO-4601, a Micromonospora secondary metabolite with a novel ring system. *J. Nat. Prod.* **2008**, *71*, 1585–1590, doi:10.1021/NP800376N.
28. Zhang, M.; Yang, C.L.; Xiao, Y.S.; Zhang, B.; Deng, X.Z.; Yang, L.; Shi, J.; Wang, Y.S.; Li, W.; Jiao, R.H.; et al. Aurachin SS, a new antibiotic from Streptomyces sp. NA04227. *J. Antibiot. (Tokyo)*. **2017**, *70*, 853–855, doi:10.1038/JA.2017.50.
29. Zhu, X.M.; Hackl, S.; Thaker, M.N.; Kalan, L.; Weber, C.; Urgast, D.S.; Krupp, E.M.; Brewer, A.; Vanner, S.; Szawiola, A.; et al. Biosynthesis of the Fluorinated Natural Product Nucleocidin in Streptomyces calvus Is Dependent on the bldA-Specified Leu-tRNA(UUA) Molecule. *Chembiochem* **2015**, *16*, 2498–2506, doi:10.1002/CBIC.201500402.
30. Zhao, G.; Yao, S.; Rothchild, K.W.; Liu, T.; Liu, Y.; Lian, J.; He, H.Y.; Ryan, K.S.; Du, Y.L. The Biosynthetic Gene Cluster of Pyrazomycin—A C-Nucleoside Antibiotic with a Rare Pyrazole Moiety. *ChemBioChem* **2020**, *21*, 644–649, doi:10.1002/CBIC.201900449.
31. Zhang, M.; Zhang, P.; Xu, G.; Zhou, W.; Gao, Y.; Gong, R.; Cai, Y.S.; Cong, H.; Deng, Z.; Price, N.P.J.; et al. Comparative Investigation into Formycin A and Pyrazofurin A Biosynthesis Reveals Branch Pathways for the Construction of C-Nucleoside Scaffolds. *Appl. Environ. Microbiol.* **2020**, *86*, doi:10.1128/AEM.01971-19.
32. Huang, S.; Tong, M.; Qin, Z.; Deng, Z.; Deng, H.; Yu, Y. Identification and characterization of the biosynthetic gene cluster of thiolutin, a tumor angiogenesis inhibitor, in Saccharothrix algeriensis NRRL B-24137. *Anticancer. Agents Med. Chem.* **2015**, *15*, 277–284, doi:10.2174/1871520614666141027145200.

33. He, J.; Magarvey, N.; Pirae, M.; Vining, L.C. The gene cluster for chloramphenicol biosynthesis in *Streptomyces venezuelae* ISP5230 includes novel shikimate pathway homologues and a monomolecular non-ribosomal peptide synthetase gene. *Microbiology* **2001**, *147*, 2817–2829, doi:10.1099/00221287-147-10-2817.
34. Ma, J.; Huang, H.; Xie, Y.; Liu, Z.; Zhao, J.; Zhang, C.; Jia, Y.; Zhang, Y.; Zhang, H.; Zhang, T.; et al. Biosynthesis of ilamycins featuring unusual building blocks and engineered production of enhanced anti-tuberculosis agents. *Nat. Commun.* **2017**, *8*, doi:10.1038/S41467-017-00419-5.
35. Tomita, H.; Katsuyama, Y.; Minami, H.; Ohnishi, Y. Identification and characterization of a bacterial cytochrome P450 monooxygenase catalyzing the 3-nitration of tyrosine in rufomycin biosynthesis. *J. Biol. Chem.* **2017**, *292*, 15859–15869, doi:10.1074/JBC.M117.791269.
36. Gonsior, M.; Mühlenweg, A.; Tietzmann, M.; Rausch, S.; Poch, A.; Süssmuth, R.D. Biosynthesis of the peptide antibiotic feglymycin by a linear nonribosomal peptide synthetase mechanism. *ChemBioChem* **2015**, *16*, 2610–2614, doi:10.1002/cbic.201500432.
37. Pelzer, S.; Süßmuth, R.; Heckmann, D.; Recktenwald, J.; Huber, P.; Jung, G.; Wohlleben, W. Identification and analysis of the balhimycin biosynthetic gene cluster and its use for manipulating glycopeptide biosynthesis in *Amycolatopsis mediterranei* DSM5908. *Antimicrob. Agents Chemother.* **1999**, *43*, 1565–1573, doi:10.1128/aac.43.7.1565.
38. Xu, L.; Huang, H.; Wei, W.; Zhong, Y.; Tang, B.; Yuan, H.; Zhu, L.; Huang, W.; Ge, M.; Yang, S.; et al. Complete genome sequence and comparative genomic analyses of the vancomycin-producing *Amycolatopsis orientalis*. *BMC Genomics* **2014**, *15*, 1–18, doi:10.1186/1471-2164-15-363.
39. Tan, G.Y.A.; Robinson, S.; Lacey, E.; Brown, R.; Kim, W.; Goodfellow, M. *Amycolatopsis regifaucium* sp. nov., a novel actinomycete that produces kigamicins. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2562–2567, doi:10.1099/IJS.0.64974-0.
40. Kaur, N.; Kumar, S.; Bala, M.; Raghava, G.P.S.; Mayilraj, S. Draft genome sequence of *Amycolatopsis decaplanina* strain DSM 44594T. *Genome Announc.* **2013**, *1*, e0013813, doi:10.1128/genomeA.00138-13.
41. Wink, J.M.; Kroppenstedt, R.M.; Ganguli, B.N.; Nadkarni, S.R.; Schumann, P.; Seibert, G.; Stackebrandt, E. Three new antibiotic producing species of the genus *Amycolatopsis*, *Amycolatopsis balhimycina* sp. nov., *A. tolypomycina* sp. nov., *A. vancoresmycina* sp. nov., and description of *Amycolatopsis keratiniphila* subsp. *keratiniphila* subsp. nov. and *A. keratiniphi*. *Syst. Appl. Microbiol.* **2003**, *26*, 38–46, doi:10.1078/072320203322337290.
42. Labeda, D.P. *Amycolatopsis coloradensis* sp. nov., the avoparcin (LL-AV290)-producing strain. *Int. J. Syst. Bacteriol.* **1995**, *45*, 124–127, doi:10.1099/00207713-45-1-124/CITE/REFWORKS.
43. Xu, F.; Wu, Y.; Zhang, C.; Davis, K.M.; Moon, K.; Bushin, L.B.; Seyedsayamdost, M.R.

A genetics-free method for high-throughput discovery of cryptic microbial metabolites. *Nat. Chem. Biol.* **2019**, *15*, 161–168, doi:10.1038/s41589-018-0193-2.

44. Yim, G.; Kalan, L.; Koteva, K.; Thaker, M.N.; Waglechner, N.; Tang, I.; Wright, G.D. Harnessing the synthetic capabilities of glycopeptide antibiotic tailoring enzymes: Characterization of the UK-68, 597 biosynthetic cluster. *ChemBioChem* **2014**, *15*, 2613–2623, doi:10.1002/cbic.201402179.
45. Pootoolal, J.; Thomas, M.G.; Marshall, C.G.; Neu, J.M.; Hubbard, B.K.; Walsh, C.T.; Wright, G.D. Assembling the glycopeptide antibiotic scaffold: The biosynthesis of A47934 from streptomyces toyocaensis NRRL15009. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 8962–8967, doi:10.1073/pnas.102285099.
46. Truman, A.W.; Kwun, M.J.; Cheng, J.; Yang, S.H.; Suh, J.W.; Hong, H.J. Antibiotic resistance mechanisms inform discovery: Identification and characterization of a novel amycolatopsis strain producing ristocetin. *Antimicrob. Agents Chemother.* **2014**, *58*, 5687–5695, doi:10.1128/AAC.03349-14.
47. Spohn, M.; Kirchner, N.; Kulik, A.; Jochim, A.; Wolf, F.; Muenzer, P.; Borst, O.; Gross, H.; Wohlleben, W.; Stegmann, E. Overproduction of ristomycin a by activation of a silent gene cluster in amycolatopsis japonicum mg417-cf17. *Antimicrob. Agents Chemother.* **2014**, *58*, 6185–6196, doi:10.1128/AAC.03512-14.
48. Sosio, M.; Kloosterman, H.; Bianchi, A.; de Vreugd, P.; Dijkhuizen, L.; Donadio, S. Organization of the teicoplanin gene cluster in Actinoplanes teichomyceticus. *Microbiology* **2004**, *150*, 95–102, doi:10.1099/mic.0.26507-0.
49. Yushchuk, O.; Vior, N.M.; Andreo-Vidal, A.; Berini, F.; Rückert, C.; Busche, T.; Binda, E.; Kalinowski, J.; Truman, A.W.; Marinelli, F. Genomic-Led Discovery of a Novel Glycopeptide Antibiotic by Nonomuraea coxensis DSM 45129. *ACS Chem. Biol.* **2021**, *16*, 915–928, doi:10.1021/acscchembio.1c00170.
50. Sosio, M.; Stinchi, S.; Beltrametti, F.; Lazzarini, A.; Donadio, S. The gene cluster for the biosynthesis of the glycopeptide antibiotic A40926 by Nonomuraea species. *Chem. Biol.* **2003**, *10*, 541–549, doi:10.1016/S1074-5521(03)00120-0.
51. Xu, M.; Wang, W.; Waglechner, N.; Culp, E.J.; Guiton, A.K.; Wright, G.D. Phylogeny-Informed Synthetic Biology Reveals Unprecedented Structural Novelty in Type v Glycopeptide Antibiotics. *ACS Cent. Sci.* **2022**, *8*, 615–626, doi:10.1021/acscentsci.1c01389.
52. Nazari, B.; Forneris, C.C.; Gibson, M.I.; Moon, K.; Schramma, K.R.; Seyedsayamdost, M.R. Nonomuraea sp. ATCC 55076 harbours the largest actinomycete chromosome to date and the kistamicin biosynthetic gene cluster. *Medchemcomm* **2017**, *8*, 780–788, doi:10.1039/c6md00637j.
53. Chiu, H.T.; Hubbard, B.K.; Shah, A.N.; Eide, J.; Fredenburg, R.A.; Walsh, C.T.; Khosla, C. Molecular cloning and sequence analysis of the complestatin biosynthetic gene cluster. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 8548–8553, doi:10.1073/pnas.151246498.

54. Banik, J.J.; Craig, J.W.; Calle, P.Y.; Brady, S.F. Tailoring enzyme-rich environmental DNA clones: A source of enzymes for generating libraries of unnatural natural products. *J. Am. Chem. Soc.* **2010**, *132*, 15661–15670, doi:10.1021/ja105825a.
55. Owen, J.G.; Reddy, B.V.B.; Ternei, M.A.; Charlop-Powers, Z.; Calle, P.Y.; Kim, J.H.; Brady, S.F. Mapping gene clusters within arrayed metagenomic libraries to expand the structural diversity of biomedically relevant natural products. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 11797–11802, doi:10.1073/pnas.1222159110.
56. Banik, J.J.; Brady, S.F. Cloning and characterization of new glycopeptide gene clusters found in an environmental DNA megalibrary. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 17273–17277, doi:10.1073/pnas.0807564105.
57. Thaker, M.N.; Wang, W.; Spanogiannopoulos, P.; Waglechner, N.; King, A.M.; Medina, R.; Wright, G.D. Identifying producers of antibacterial compounds by screening for antibiotic resistance. *Nat. Biotechnol.* **2013**, *31*, 922–927, doi:10.1038/nbt.2685.
58. Lei, X.; Yuan, F.; Shi, Y.; Li, X.; Wang, L.; Hong, B. Draft genome sequence of norvancomycin-producing strain *Amycolatopsis orientalis* CPCC200066. *Genome Announc.* **2015**, *3*, doi:10.1128/genomeA.00296-15.
59. Waglechner, N.; McArthur, A.G.; Wright, G.D. Phylogenetic reconciliation reveals the natural history of glycopeptide antibiotic biosynthesis and resistance. *Nat. Microbiol.* **2019**, *4*, 1862–1871, doi:10.1038/s41564-019-0531-5.
60. Liu, K.; Hu, X.R.; Zhao, L.X.; Wang, Y.; Deng, Z.; Taoa, M. Enhancing Ristomycin A Production by Overexpression of ParBLike StrR Family Regulators Controlling the Biosynthesis Genes. *Appl. Environ. Microbiol.* **2021**, *87*, 1–19, doi:10.1128/AEM.01066-21.
61. Khatri, I.; Subramanian, S.; Mayilraj, S. Genome sequencing and annotation of *Amycolatopsis azurea* DSM 43854T. *Genomics Data* **2014**, *2*, 44–45, doi:10.1016/j.gdata.2013.12.003.
62. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027, doi:10.1093/molbev/msab120.
63. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Meng, E.C.; Couch, G.S.; Croll, T.I.; Morris, J.H.; Ferrin, T.E. UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci.* **2021**, *30*, 70–82, doi:10.1002/pro.3943.
64. Mirdita, M.; Schütze, K.; Moriwaki, Y.; Heo, L.; Ovchinnikov, S.; Steinegger, M. ColabFold: making protein folding accessible to all. *Nat. Methods* **2022**, *19*, 679–682, doi:10.1038/s41592-022-01488-1.
65. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021**, *596*, 583–589,

doi:10.1038/s41586-021-03819-2.

66. Sievers, F.; Higgins, D.G. Clustal Omega. *Curr. Protoc. Bioinforma.* **2014**, 2014, 3.13.1-3.13.16, doi:10.1002/0471250953.bi0313s48.