ELECTRONIC SUPPLEMENTARY INFORMATION

Unlocking the biosynthetic regulation role of polyketide alkaloid lydicamycins

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1. Material and methods

1a. Generating *Streptomyces ginsengnesis* G7 mutants. Genomic DNA from *Streptomyces ginsengnesis* G7 was isolated using the Hipure Bacterial DNA Kit (Magen). Fragments necessary for cloning were amplified via PCR using Phanta Max Super-Fidelity DNA Polymerase (Vazyme). The plasmid pIB139 was digested with *Nhe*I restriction enzyme and NEB buffer, following the manufacturer's instructions. Multiple DNA fragments were assembled into the digested vector backbones using the Trelief Seamless Cloning Kit (Tsingke). The final vector was propagated in *E. coli* DH5α, and recombinants were verified through Sanger sequencing. Conjugation experiments were performed to introduce the final vector into *Streptomyces ginsengnesis* G7, using *E. coli* ET12567/pUZ8002 as previously described[1]. Ex-conjugants were collected from the conjugation plates after incubation for 7 days under apramycin and nalidixic acid overlay. Subsequently, they were transferred to new plates and allowed to grow for an additional 7 days under apramycin selection. Single colonies resulting from this process were replica-plated onto apramycin-containing plates for phenotype screening and mutant verification by PCR.

1b. Lydicamycin congener analysis. Cultures of *Streptomyces ginsengnesis* wild-type and mutant strains were cultivated on International Streptomyces Projects 2 (ISP2) agar plates at a temperature of 30 °C. From each plate, three equally sized agar plugs (1 cm^3) were extracted and vigorously shaken with 500 μL of methanol for 5 minutes. Following this, the samples were centrifuged twice at 15,000 rpm for 5 minutes each time. The resulting extracts were subjected to High-Performance Liquid Chromatography (HPLC) analysis using the Agilent 1290 Infinity II system. The following chromatographic method was used throughout this work: Phenomenex

Kinetex C_{18} column (100 × 2.1 mm, 100 Å); mobile phase A: water + 0.1% formic acid; mobile phase B: acetonitrile + 0.1% formic acid. Elution gradient: 0–1 min, 20% B; 1–12 min, 20%–100% B; 12–14 min, 100% B; 14–14.1 min, 100%–20% B; 14.1–17 min, 20% B; flow rate 0.3mL/min; injection volume 10 μL.

1c. Molecular networking analysis. A molecular network was created using the online workflow (https://ccms-ucsd.github.io/GNPSDocumentation/) on the GNPS website (http://gnps.ucsd.edu)[2]. The data was filtered by removing all MS/MS fragment ions within +/- 17 Da of the precursor m/z. MS/MS spectra were window filtered by choosing only the top 6 fragment ions in the +/- 50Da window throughout the spectrum. The precursor ion mass tolerance was set to 0.1 Da and a MS/MS fragment ion tolerance of 0.1 Da. A network was then created where edges were filtered to have a cosine score above 0.1 and more than 6 matched peaks. Further, edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS' spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks.

2. ESI Tables

ESI Table 1. Strains, plasmids and primers used in this work.

Strain name	**Description**	**Source or Ref.**
E. coli ET12567	*dam- dcm- hsdS-*	[3]
E. coli DH5α	F– φ80*lac*ZΔM15Δ(*lacZYA-argF*) *U169* *rec*A1 *end*A1 *hsd*R17 (rK– mK+) *pho*A *sup*E44 λ- *thi*–1 *gyr*A96 *rel*A1	Tsingke Biotechnology Co., Ltd., China
Streptomyces ginsengnesis G7	Wild-type strain	This work
Streptomyces ginsengnesis G7*_Δlyd67*	Mutant stain in which the LuxR regulation gene was deleted.	[4]
Streptomyces ginsengnesis G7*_lyd67*	Mutant stain in which the LuxR regulation gene was overexpressed.	This work
Plasmids and ePACs	**Description**	**Source or Ref.**
pIB139	pIB139 is used to overexpress a gene	From Professor Yu Yi
pUZ8002	Non-transmissible RK2 derivative with a mutation in *oriT*	[3]

Primer name	**Sequence**	**Description**
ctg1_1131-fwd	ggttggtaggatccacatatgATGGGTGGCTCAGAGCAGG	To amplify *Lyd11* gene
ctg1_1131-rev	ctcgcccttgctCACGCTCTCTCCGCGGAGGGT	
ctg1_1152-fwd	ggttggtaggatccacatatgGTGATCAAGGTTCTCGTCACGGATG	To amplify *Lyd32* gene
ctg1_1152-rev	ctcgcccttgctcacGGTGCCCAGGCCGGCG	
ctg1_1153-fwd	ggttggtaggatccacatatgGAGCGGGTGAGGCGCGC	To amplify *Lyd33* gene
ctg1_1153-rev	ctcgcccttgctcacCGCCTTGACCTCGAAGGACTTCTTCT	
ctg1_1154-fwd	gtgccggttggtaggatccacatatgAGTCCCAAGGCCATGGCCC	To amplify *Lyd34* gene
ctg1_1154-rev	agctcctcgcccttgctcacGTCGGGCGAGACGGGTGAG	
ctg1_1163-fwd	ggttggtaggatccacatatgGTGCCGCGTCCGGAGCAG	To amplify *Lyd43* gene
ctg1_1163-rev	ctcgcccttgctcacCCGGCTGAGGCCGCTCG	
ctg1_1184-fwd	ggttggtaggatccacatatgGTGCGTGTTGTCCTCGTAGAGG	To amplify *Lyd64* gene

ctg1_1184-rev	ctcgcccttgctcacGTCCAGGTTCGCGCTGTTC	
ctg1_1185-fwd	ggttggtaggatccacatatgTTCGGACGGCACCTGTGG	To amplify *Lyd65* gene
ctg1_1185-rev	ctcgcccttgctcacCGAGGACAACACGCACGG	
ctg1_1187-fwd	ggttggtaggatccacatatgGGGTTAGTGGAACGTGACGCGG	To amplify *Lyd67* gene
ctg1_1187-rev	ctcgcccttgctcacGGCGATATCCGCGATGTCCGC	

ESI Table 2. The media used in this work.

Media	**Recipe (per litre)**	**Water**	**pH**
LB	10 g tryptone 5 g yeast extract 10 g NaCl +/- 20 g agar	Deionised	7.5
MS	20 g soy flour 20 g mannitol 20 g agar	Tap	As made
ISP2	4 g glucose 4 g yeast extract 10 g malt extract 10g agar	Tap	7.2

ESI Table 3. The antibiotics and concentrations used in this work.

Antibiotic	**Final concentration used for selection (μg/ml)**
Apramycin	50/25
Chloramphenicol	25
Nalidixic Acid	25

3. ESI References

1. T. Kiese, M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood, *Practical Streptomyces Genetics*, 2000.

2. M, Wang, et al. *Nat Biotechnol*. 2016, **34**, 828-837.

3. A. C. Jones, B. Gust, A. Kulik, L. Heide, M. J. Buttner and M. J. Bibb, *PLoS ONE*, 2013, **8**, e69319.

4. H. Zhang, X. Li, S. Pan, J. Huang and Z. Qin, *New J. Chem.*, 2023, **47**, 12093-12100.