Supporting Information

An Amidinohydrolase Provides the Missing Link in the Biosynthesis of Amino Marginolactone Antibiotics

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Supporting Information

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1.1. Bacterial strains and culture conditions

*Streptomyces olivaceus* Tü4018 (desertomycin and kanchanamycin-producing strain) was the kind gift of Pr. Dr. Wolfgang Wohlleben, University of Tübingen. *Streptomyces macronensis* Dietz sp. nov. UC 8271 (NRRL 12566) and *Streptomyces spectabilis* NRRL B2494 (desertomycin-producing strains) were obtained from the Agricultural Research Service Culture Collection, Peoria, USA. *Saccharomonospora azurea* (syn. *S. caesia*) DSM 43044 (primycin-producing strain) and *Streptomyces violaceusniger* DSM 4137 (azalomyacin-producing strain) were obtained from the Leibnitz Institut - DSMZ. All strains were maintained on SFM agar (2% soya flour (AYKASOY), 2% D-mannitol, 2% agar) at 30°C. *E. coli* strains were grown in Luria-Bertani (LB) broth (10% tryptone, 5% yeast extract, 10% NaCl) or agar (10% tryptone, 5% yeast extract, 10% NaCl, 2% agar) at 37°C with appropriate antibiotic selection (kanamycin, at 50 µg ml⁻¹).

1.2. Materials, DNA isolation and manipulation

Bacterial strains, plasmids and oligonucleotides (Eurofins) used in this work are summarized in Tables S1, S2 and S3 respectively. Restriction endonucleases were purchased from New England Biolabs (NEB). T4 DNA ligase and alkaline phosphatase were purchased from Fermentas. All chemicals were from Sigma-Aldrich. Liquid cultures for isolation of genomic DNA were grown in tryptone soya broth (Difco). DNA isolation and manipulation in *Streptomyces*, and *E. coli* were carried out using standard protocols.[1,2] PCR amplifications were carried out using Phusion® High-Fidelity DNA Polymerase (NEB). *E. coli* BL21(DE3) (Novagen) was used for protein expression.

1.3. Metabolite analysis and compound isolation

For small-scale analysis, *Streptomyces macronensis*, *Saccharomonospora azurea*, and *Streptomyces violaceusniger* DSM 4137 strains were grown in liquid TSBY medium (3% TSB (Tryptic Soy Broth), 10.3% sucrose, 0.5% yeast extract) at 30°C and 150 rpm in a rotary incubator for 2-3 days. *Streptomyces olivaceus* Tü4018 was grown in GYM medium (0.4% glucose, 0.4% yeast extract, 1% malt extract, pH 7.2) for 2-3 days. 1 mL samples of culture broth were centrifuged at 20,000 x g for 15 min. The mycelia pellets were then extracted with 1 mL of methanol at 60°C for 2 hours. The mixture was spun down and the clear methanol extract was evaporated to dryness and dissolved in 200 µL of methanol. 10 µL of the extract was analyzed by LC-MS. LC-MS analyses were performed on a HPLC (Agilent Technologies 1200 series) coupled to a Thermo Fisher LTQ mass spectrometer fitted with an electrospray ionization (ESI) source. For extracts from *Streptomyces macronensis* and from *Streptomyces olivaceus* Tü4018, a Luna 5µ C18 column (2.0 x 250 mm, Phenomenex) was used, and the samples were eluted using MQ containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic
acid (B) at a flow rate of 0.2 ml min⁻¹. The linear elution gradient for extracts from *Streptomyces macrornensis* was 25% to 50% B over 20 min, 50% to 100% B over 9 min. The elution gradient for extracts from *Streptomyces olivaceus* Tü4018 was 25% to 50% B over 15 min, 50% to 75% B over 30 min, 75% to 100% B over 4 min. For extracts from *Saccharomonospora azures* and from *Streptomyces violaceusniger* DSM 4137, a Prodigy 5µ C18 column (4.6 x 250 mm, Phenomenex) was used, and the samples were eluted using MQ containing 20mM ammonium acetate (A) and methanol (B) at a flow rate of 0.7 ml min⁻¹. The elution gradient for both extracts was 60% to 95% B over 30 min. The mass spectrometer was run in positive ionization mode, scanning from m/z 200 to 2000 in full scan mode. MS/MS analysis were performed on [M+H]⁺ ions with a normalized collision energy of 30%. High-resolution mass analysis was carried out on Thermo Fisher Orbitrap mass spectrometer with resolution set up at 60 K.

For desertomycin B production and isolation, six 250 ml Erlenmeyer flasks with spirals, containing 50 ml TSBY medium, were inoculated with 1 ml 2-day TSBY seed culture of *S. macrornensis* dstH-deletion mutant, and incubated at 30 °C, 200 rpm. After 3 days, the broth was centrifuged at 9,000 rpm for 20 min. The pellet was resuspended in methanol and incubated at 60 °C for 2 h. The methanol extract of mycelia pellets was evaporated to dryness under reduced pressure with the rotary evaporator. The residue was dissolved in methanol, and desertomycin B was purified from a preparative HPLC (Agilent 1200) fitted with a Luna C18 column (100Å, 21.20 x 250 mm, Phenomenex). Compounds were eluted with MQ containing 0.1% formic acid (A) and MeCN containing 0.1% formic acid (B) with a linear gradient of 5% to 35% B over 10 min, 35% to 65% B over 15 min, 65% to 100% B over 10 min at a flow rate of 20 ml/min. Fractions were collected, and checked by MS analysis. Fractions containing desertomycin B were combined. Acetonitrile was removed under reduced pressure, and sample was lyophilized.

For kanchanamycin C production and isolation, six 1 L Erlenmeyer flasks with spirals, containing 250 ml GYM medium, were inoculated with 2.5 ml 2-day GYM seed culture of *Streptomyces olivaceus* Tü4018 and incubated at 30 °C, 200 rpm. After 6 days, the broth was centrifuged at 9,000 rpm for 20 min. The pellet was resuspended in methanol and incubated at 60 °C for 2 h. The suspension was centrifuged in Falcon tubes at 2,500 rpm for 15 min. The supernatants were combined and filtered into a 1 L round flask. The methanol was removed under reduced pressure with the rotary evaporator to give a yellowish residue. The residue was extracted two times with diethyl ether/water. The diethyl ether was removed with the rotary evaporator. After lyophilisation the residues were dissolved in methanol for purification by preparative HPLC. Compounds were eluted with 5 mM ammonium acetate (A) and methanol (B) with a linear gradient of 60% B to 95 % B over 30 min, at a flow rate of 20 ml/min. Fractions were collected, and checked by MS analysis. Fractions containing kanchanamycin C were combined. After removing the methanol under reduced pressure, sample was lyophilized.

For primycin A1 production and isolation, 1 ml 2-day TSBY seed culture of *S. caesia* was inoculated into 100 ml inoculum medium[3] (3% soya flour, 5% wheat starch, 2% NaCl, 0.75% CaCO₃,
0.5% Sunflower oil) in a 500 ml Erlenmeyer flask with spiral at 30 °C, 240 rpm. After 2 days, five 500 ml Erlenmeyer flask, containing 100 ml of fermentation medium (5% soya flour, 5% wheat starch, 2% NaCl, 0.75% CaCO₃, 0.6% sunflower oil, 0.4% stearic acid, 0.1% KH₂PO₄), were inoculated with 10 ml inoculum medium and cultivated for 48 h at 30 °C and 240 rpm. Under this fermentation conditions, primycin A1 became the major component. The cultures were centrifuged at 9,000 rpm for 20 min. The pellet was resuspended in methanol and incubated at 60 °C for 2 h. The methanol extract of mycelia pellets was evaporated to dryness under reduced pressure with the rotary evaporator. The residue was dissolved in MeOH, and was purified by solid phase extraction using ISOLUTE® C18 (EC) SPE columns. Primycin A1 was eluted with 60 % acetonitrile/ 40 % milliQ water. After removing the acetonitrile under reduced pressure, sample was lyophilized.

For azalomycin F4a production and isolation, six 1 L Erlenmeyer flasks with spirals, containing 250 ml TSBY medium, were inoculated with 2.5 ml 2-day TSBY seed culture of Streptomyces violaceusniger DSM 4137 and incubated at 30 °C, 200 rpm. After 3 days, the broth was centrifuged at 9,000 rpm for 20 min. The pellet was resuspended in methanol and incubated at 60 °C for 2 h. The methanol extract of mycelia pellets was evaporated to dryness under reduced pressure with the rotary evaporator. The residue was dissolved in MeOH, and separated on a sephadex LH20 column with MeOH/chloroform (1:1). The fractions were checked by MS. Fractions containing azalomycin F4a were combined, and solvents were removed under reduced pressure. The residue was dissolved in MeOH, and further purified by semi-preparative HPLC on a Prodigy C18 column (10 x 250 mm, Phenomenex) with a linear gradient of 45% MeCN, 55% 5 mM ammonium acetate to 56% MeCN, 44% 5 mM ammonium acetate over 35 minutes with a flow rate of 10 ml/min. Fractions containing azalomycin F4a were combined. Acetonitrile was removed under reduced pressure, sample was lyophilized.

1.4. Gene knock-out in S. macronensis

The amidinohydrolase gene dstH in S. macronensis was knocked out by in-frame deletion. To construct the deletion plasmid pYH7-dstH, dstH upstream and downstream fragments (about 2 kb) were amplified from S. macronensis genomic DNA by PCR with primers dstH-up F, dstH-up R and dstH-dn F, dstH-dn R, respectively. The cloning vector pYH7⁴ was digested with NdeI, treated with shrimp alkaline phosphatase (SAP) and gel purified. To ligate the two fragments into pYH7, the isothermal assembly method was used as described.⁵ The mixture was incubated at 50°C for 60 min, and then was used to transform E. coli DH10B. The integrity of the plasmid was checked by restriction digestion and sequencing.

The construct was then introduced by conjugation into S. macronensis. The donor strain was E. coli ET12657/pUZ8002, and conjugation was carried out on 20 ml of SFM plates (2% mannitol, 2% soya flour, 2% agar). After incubating at 30°C for 20 hours, exconjugants were selected with 50 µg ml⁻¹ apramycin and 25 µg ml⁻¹ nalidixic acid. Single colonies from this plate were transferred to a SFM plate
containing 50 µg ml\(^{-1}\) apramycin to double check for antibiotic resistance. Mutant screening was carried out by streaking transformants on SFM agar medium for non-selective growth, then patching single colonies onto both SFM agar and SFM agar containing apramycin (50 µg ml\(^{-1}\)) in parallel. Candidate colonies with the correct phenotype (Apr\(^{S}\)) were selected for further screening by PCR with a pair of primers dstH-CP1 and dstH-CP2 to identify double cross-over mutants. The PCR fragments from the double cross-over mutants were further verified by sequencing.

1.5. Protein expression and purification

The dstH gene was amplified by PCR, using genomic DNA of *Streptomyces olivaceus* Tü4018 as template, and inserted into vector pET28a via *NdeI* and *HindIII* restriction sites to yield pET28a-dstH. The identity of the plasmid was confirmed by DNA sequencing.

The pET28a-dstH was then used to transform *E. coli* BL21(DE3) for protein expression. A single colony was inoculated into 10 mL of LB medium containing 50 µg ml\(^{-1}\) kanamycin and grown overnight at 37°C, 250 rpm. An aliquot (1 mL) was retained for preparation of a glycerol stock and the remaining culture was inoculated into 1 L LB medium containing 50 µg ml\(^{-1}\) kanamycin and incubated at 37°C, 200 rpm until A\(_{600}\) reached 0.6 before addition of 400 µL of 1 M isopropyl-β-D-thiogalactopyranoside (IPTG) and incubation at 22°C overnight to induce protein expression. Cells were harvested by centrifugation at 4,000 rpm for 10 min, resuspended in lysis buffer (20 mM Tris-HCl, pH 7.8, 0.5 M NaCl, 10 mM imidazole) and lysed by sonication. The total lysate was centrifuged at 14,000 rpm for 40 min, and the supernatant was loaded onto a His-Bind column (1 mL bed volume), which had been pre-charged with nickel ions and equilibrated with lysis buffer. The column was washed with 10 column volumes of lysis buffer. Bound proteins were then eluted with a step gradient of increasing imidazole concentration (40, 80, 100, 150, 200, 250 and 500 mM in binding buffer). The protein solutions were concentrated, and further purified by gel filtration on an ÄKTA Explorer FPLC system fitted with a HiLoad 16/60 Superdex 200 Prep Grade column. The mobile phase contained 100 mM potassium phosphate, pH 7.4. Fractions containing protein of the expected size were pooled and concentrated using Amicon Ultra-4 concentrators (Millipore) fitted with a 30 kDa filter. All purification steps were carried out at 4°C. The purity of the protein was examined by 4 - 12% Bis-Tris Gel (Novex) analysis and the concentration of the protein was measured by Bradford assay using bovine serum albumin as a standard.

1.6. *In vitro* activity assays of DstH

Each reaction mixture (25 µl) contained 5 µM purified DstH, 1 mM CoCl\(_2\) (or NiCl\(_2\), MnCl\(_2\), ZnCl\(_2\), MgCl\(_2\), MQ as no-metal control), in 50 mM Tris-HCl buffer pH 9.0. After incubation at 37°C for 30 min, 0.5 µl of purified desertomycin B (or primycin A1, kanchanamycin C, azalomycin F4a) stock solution (in DMSO) was added to a final concentration of 0.3 mM, and the reaction was allowed
to continue at 37°C for 3 hr. 10 µl of the reaction mixture was taken, mixed with 50 µl methanol, and analyzed by HPLC-MS with a Luna 5µ C18 column (2.0 x 250 mm, Phenomenex) eluting with MQ containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) at a flow rate of 0.2 ml min⁻¹. The linear elution gradient for assays when desertomycin B or primycin A1 was used as substrate was 25% to 50% B over 20 min, 50% to 100% B over 9 min. The elution gradient for assays when kanchanamycin C was used as substrate was 25% to 50% B over 9 min, 50% to 72% B over 26 min, 72% to 100% B over 5 min. The elution gradient for assays when azalomycin F4a was used as substrate was 25% to 50% B over 5 min, 50% to 75% B over 20 min, 75% to 100% B over 5 min.

2. Supplementary Scheme and Figures

![Scheme S1](image)

**Scheme S1. Conserved genes in the desertomycin and primycin biosynthetic gene clusters.** The putative amidinohydrolases encoded by genes *dstH* and *priH* are highlighted. PKS, polyketide synthase multienzymes; AM, arginine 2-mono-oxygenase; AH, 4-guanidinobutyramide hydrolase; CoL, 4-guanidinobutanoate:CoA ligase; AT, 4-guanidinobutyryl-CoA:ACP acyltransferase; ACP, acylcarrier protein; TEII, discrete thioesterase.
Figure S1: Polyketide synthase (PKS) domain organisations of biosynthetic gene clusters for desertomycin in *Streptomyces macrocinus* (or in *Streptomyces spectabilis* NRRL B-2494, or in *Streptomyces olivaceus* Tü4018), primycin in *Saccharomonospora azurea* DSM 43044, kanchanamycin in *Streptomyces olivaceus* Tü4018 and azalomycin in *Streptomyces violaceus* DSM 4137.

## Azalomycin

| Name  | Sequence                                                                 | Source                  |
|-------|---------------------------------------------------------------------------|-------------------------|
| KR14  | HAAGVTLAASLLETLELADAATTVSGKVAAGAVNLDELLGDRELDFAVFSSISGVWGGSGQGVYGSNAPFLD | *Streptomyces macr. (...)* |
| KR9   | HAAGVQAEALAMGLADAASVSGKATAGHLDDLGDRELDFAVFSSIAAVGWSGGQQAAYGAANAYLD       | *Streptomyces spectabilis* NRRL B-2494 |
| KR7   | HAAGVQAEALAMGLADAASVSGKATAGHLDDLGDRELDFAVFSSIAAVGWSGGQQAAYGAANAYLD       | *Streptomyces spec. (...)* |
| KR11  | HAAGANAAPLETTVADAANAGAVGNLDELLGDRELDFAVFSSIAAVGWSGGQQAAYGAANAYLD         | *Streptomyces spec. (...)* |
| KR1   | HAAGVLDGVIDTSLSPKIDAVFPEKVIDAANWLHELTRTLDDLAEFVMMGSSGQGNAYAANAYLD         | *Streptomyces spec. (...)* |
| KR4   | HTAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR3   | HTAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR15  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR8   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR10  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR12  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR6   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR17  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR18  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR2   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR5   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR1   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR4   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR13  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR17  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |

## Kanchanamycin

| Name  | Sequence                                                                 | Source                  |
|-------|---------------------------------------------------------------------------|-------------------------|
| KR14  | HAAGVTLAASLLETLELADAATTVSGKVAAGAVNLDELLGDRELDFAVFSSISGVWGGSGQGVYGSNAPFLD | *Streptomyces macr. (...)* |
| KR7   | HAAGVQAEALAMGLADAASVSGKATAGHLDDLGDRELDFAVFSSIAAVGWSGGQQAAYGAANAYLD       | *Streptomyces spectabilis* NRRL B-2494 |
| KR11  | HAAGANAAPLETTVADAANAGAVGNLDELLGDRELDFAVFSSISGVWGGSGQQAAYGAANAYLD         | *Streptomyces spec. (...)* |
| KR1   | HAAGVLDGVIDTSLSPKIDAVFPEKVIDAANWLHELTRTLDDLAEFVMMGSSGQGNAYAANAYLD         | *Streptomyces spec. (...)* |
| KR4   | HTAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR3   | HTAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR15  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR8   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR10  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR12  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR6   | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |
| KR17  | HAAGVLDGVIDTLETFPDRELPKVIDAANWLHELTAGLDDLAFVLSLGSQGQNYAANAYLD             | *Streptomyces spec. (...)* |

## Desertomycin
KR17  HVAVGDVGVTALTLPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR20  HVAVGDVGVTALTLPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR12  HVAGALGDGVTALTLPERALVLPKDAAVNHLGATAGL-----NLHAFVLSAAGVGFPGQANYAANFLD B1
KR3   HVAGVLGDGVTALTLPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR14  HVAVLDGVDGVTALTLPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR16  HVAVLDGVDGVTALTLPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR6   HTAGVFDGITASLTPERLATVFRAKADARVNHELTRDG---EHLDFAVLSAAGVGFPGQANYAANFLD B1
KR21  HTAGVLDALVSLTPERALVLPKDAAVNHLGATAGL-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR1  HAAGVLDDGVVDGLTPERLATVFRAAGAADVLHELTRDLD-----DLSAFVLSAAGVGLGSQAANYAANFLD B1
KR11  HAAGVLDDGVLDAMTPQRLATVFRAASEARNLDELTADDLSLFVSSSAGVGFPGQANYAANFLD B1
KR17  HTAGTLDDGVLDNLTPERVSTVLRSKVDGAVHLDELTREDSLAFVLFSSASGVFGGQPANYAANFLD B1
KR4   HAAGVLDDGVVSMTAQRIETVMAPKALAAWNLHELTRDHDVAFVLFSSASGVFGGQPANYAANFLD B1
KR13  HTAGVLDDGVLIDLTPERLATVFRAKVESARHLDELTRDA---DLDAFVLSAAGVGLGSQAANYAANFLD B1
KR5   HAAGVLDDGVLDGLTAPDRLDGVLRPKSPAATALHELTRDLTAFVLFSSVAGTVGGAGVANYAAANAFLD B1
KR19  HAAGVLDDGVLDGLTAPDRLDGVLRPKSPAATALHELTRDLTAFVLFSSVAGTVGGAGVANYAAANAFLD B1
KR10  HAAGVSPALADTDTPADLAVLRPKSAATALHELTRDLTAFVLFSSIAAVWGSGGQAGYAAANAFLD A1
KR13  HAAGIGQTQPLDGMGVADIAEVFGAKTAGAAHLDDLLGAD---DLDAFVLSAAGVGLGSQAANYAANFLD B1
KR12  HAAGIAQSTPLVCDSVEEVAEVAVGKAVANHLHELTDL-----DLDAFVLSAAGVGLGSQAANYAANFLD A1
KR16  HAAGMAQSTALVDCSVEEVAEVAVGKAVANHLHELTDL-----DLDAFVLSAAGVGLGSQAANYAANFLD A1

Figure S2: Sequence alignment of the PKS KR domains. The active site residue Y is marked with an asterisk. The arrows indicate the residue predictive of B and A-type alcohol stereochemistry, respectively. The predicted configurations of the α- and β-stereocenters generated by each KR, according to the model of Keatinge-Clay,\textsuperscript{[6]} are indicated to the right of the alignment. A1: 2R, 3S; A2: 2S, 3S; B1: 2R, 3R; B2: 2S, 3R.
Figure S3: Predicted linear structures for azalomycin, kanchanamycin, primycin and desertomycin based on the bioinformatic analysis of ketoreductase (KR) domain. The predicted configuration for desertomycin at C32-methyl (highlighted in blue) is opposite from the configuration established by Kishi et al.\(^7\), all the other 20 stereocenters have the same configurations as those established by Kishi and colleagues.
Figure S4. HPLC-MS analysis of desertomycin production in *S. macronensis*. a) LC-MS chromatogram of desertomycin A and desertomycin B. b) ESI-MS spectrum of desertomycin A ([M+H]^+: 1192.7; [M+Na]^+: 1214.7) and desertomycin B ([M+H]^+: 1234.7). c) ESI-MS/MS spectra of desertomycin A ([M+H]^+: 1192.7) and desertomycin B ([M+H]^+: 1234.7).
Figure S5. HPLC-MS analysis of primycins in Saccharomonospora azurea. a) LC-MS chromatogram of various primycins. b) ESI-MS spectrum of primycins. c) ESI-MS/MS spectra of deguanidino-amino-primycin A1 ([M+H]+: 1036.7) and primycin A1 ([M+H]+: 1078.7). The isomers for 2A, 2G, 3A and 3G likely represent structural isomers of side-chain R1.
**Figure S6. HPLC-MS analysis of desertomycins and kanchanamycins in Streptomyces olivaceus Tü4018.** a) LC-MS chromatogram of desertomycin A, desertomycin B and kanchanamycins. b) ESI-MS spectra of desertomycin A, desertomycin B and kanchanamycins. c) ESI-MS/MS spectra of deguanidino-amino-kanchanamycin C ([M+H]+: 1012.7) and kanchanamycin C ([M+H]+: 1054.7). The two peaks for deguanidino-amino-kanchanamycin C at 18.0 min and 20.2 min are probably isomers, the same is true for kanchanamycin C at 19.1 min and 21.4 min. The two isomers, by analogy with azalomycin, are likely due to a different site of attachment of the malonyl group, either at C23-OH or at C25-OH.[8]
**Figure S7. HPLC-MS analysis of azalomycin F4a in Streptomyces violaceusniger DSM4317.**

(a) LC-MS chromatogram of azalomycin F4a. (b) ESI-MS spectrum of azalomycin F4a. (c) ESI-MS/MS spectrum of azalomycin F4a ([M+H]+: 1082.7). The two peaks for azalomycin F4a at 23.0 min and 24.6 min are isomers, which are due to different attachment site of malonyl group, one is at C23-OH and the other is at C25-OH. 

A)

| 10 | 20 | 30 | 40 | 50 | 60 |
|----|----|----|----|----|----|
| AMH_A828 | --MSETPEAEAWREVRDSTFPFERGPDLLRYYVQYSVGPTFMGVPFLAITEQDRLA |
| GbuA_PA | VPKHDDQPHASSYPNQKAS---RDPGLNPHRANQAPAXYVGIPFTMGLPCLTPEDLRA |
| GpuA_PA | -----------------------------------NLHPPGKNM---MEPRFGGIATMRMLPHQPSAKD |
| PAH_SC | -----------------------------------PQPLDAAE---IPRFGIPTFMRLLPHTDQPR--- |
| Agm_BT | ----------------------------------- ----SPRYAQTIFMRLLPHDPOPR---G |
| Agm_DR | ----------------------------------- --------YGGIPTQAPLVPQDGQWQA |
| Agm_CD | ----------------------------------- ----LNY |
| Agm_TV | ----------------------------------- |
| ARG_BC | ----------------------------------- |
| ARG_TT | ----------------------------------- |

| 71 | 80 | 90 | 100 | 110 | 120 |
|----|----|----|----|----|----|
| AMH_A828 | GEVDVAVVGCPTVVSSGHR---GAYGPRARADERLYATPEGFVHSTRVNNPFLIKVVD |
| AMH_A821 | GVGDVAVLGAPVDTSTGHR---GAFGPRALDERLYFNNTSDLVNASTRKIPFDELTVGD |
| GbuA_PA | ----LDAAEFGVPLDGI7LRSFTRFSGPREEASVLMVRR-----YNMAAAGAPDSLNVAD |
| GpuA_PA | ----LQVGLGVPWDFGGTNRAGHRPVEVRLSSLFLMRK---VHVSRIAPYLVRVGD |
| PAH_SC | ----YDVVVGAAPYDGTGTSYPRGARFQPQAIRSESLGIGH---VGCQGFPGTDVLNCCD |
| Agm_BT | ----PLDLATTFRSKGLOPSAVRAASVQLAB---LNFPWNGFDPFDLVDVAD |
| Agm_DR | ----DVALQPISAFLPGRFQAPARALREALSLSRSP---FTGLOKRLQGVTFAD |
| Agm_CD | EESNLDVGPVDGTDSSNMPCARRASSKREKYGLET------YSPFPLLDDLDVYNCD |
| Agm_TV | -------VGFIPPPNTSRYRSGKYAPSNGAYVNLE---YEYSYGIDLASSGAXD |
| ARG_BC | -------ISIIGVMPDLGQT---RVRDMGSPSAMRMYAVGIERLHLYHDELGDIP---KAER |
| ARG_TT | -------VAVVGVPMDDLGN---RVRDMGSPSALRYARLLEQLEDGVTELGDVPSVLARASR |

| 130 | 140 | 150 | 160 | 170 | 180 |
|-----|-----|-----|-----|-----|-----|
| AMH_A828 | YGDAAVDFPDTIERSMEPIKGLVREIAE---GARFVVLGSLHPLPSVGALSEVHGRG |
| AMH_A821 | YGDAAVDLSIENGERTIGQVSEVLVD---GAPVLMKGGGRMVNMVTALVEKYGD |
| GbuA_PA | IGDCAVITMNLLEVAVRIEQEYDRLG---GILPLGSGNITLPILAIITXHG--- |
| GpuA_PA | LGDPVNFIPDLDSLRLJERGYPQVHA---GTLPLGSGNITLPILAIRGRPRP-- |
| PAH_SC | AGDINLPFDMNAIAIDTAGSLSLGLKKA---NAALMJGDEPLTVLARVAEIQHG-- |
| Agm_BT | YGDCWFDADHPSLASKPAIVHENARTIQS---DAMLDIGSGEITYPLIIIAHAYQGYK-- |
| Agm_DR | AGDVILIPSSPLQLAHRIESTEAARQVRGR---CRVFPGDSLPSVPLRAFADVPD-- |
| Agm_CD | YGDELEISVGSTQVEVKEIYEQTYIKVRD---SKVFXIGEGRLVPAFVAKVHEKYN-- |
| Agm_TV | LGDEESEE-DEYVYIDDVESVSTAVAXSD---GKIPXLEGESITVTGAVRILPK--- |
| ARG_BC | LHEQGDSRNLRAKAWAEKNAAXADVQVRGFRPFLVGDSRAIAIGTLAAGVAKHYE-- |
| ARG_TT | RRGRLAYLEEIRAAIRVRKLVLRALAAPE---GVFFIVLGLSMGVSAGAARG-R--- |

| 190 | 200 | 210 | 220 | 230 | 240 |
|-----|-----|-----|-----|-----|-----|
| AMH_A828 | SIAVIHSGDKPPHEEELFGR-ATHTPPIRRLIDE----MVPGRNVIQVG |
| AMH_A821 | KLVAVGHSNFHPPCHEEYIOHTKHTAIIWLVNL---VQPVNGHVQAGI |
| GbuA_PA | XVGVHLHARADAONHMFGX---IAKTTFRAVEED---LLDCRQTVIGL |
| GpuA_PA | -LGVRHAIHDNSNPAFGYVR-YHGTKFRHGIDEK----LIDPAAMVQGI |
| PAH_SC | PIAYIAHDSNPAYFGYR-YHGTKFRHGIDEK----LIDPAAMVQGI |
| Agm_BT | PLSLIRHACWHDNADFPADLS-LHMGTMFYAVKDQ---LIDPKASQVIG |
| Agm_DR | ----LHVCQIALIDIDTRN-NZKWSNSPPFRAC-EA---LNPVNLHITTVO |
| Agm_CD | ----DVTYVHARCAKIDZEEYNNKS-NHATVIRKIDW---TVGDNKIPIFGI |
| Agm_TV | DDDVLVHARCAKIDZEEYNNKS-NHATVIRKIDW---TVGDNKIPIFGI |
| ARG_BC | RLGVWIRAHGHDNTAETSPSGNHICPMLAASLGFGHAPLTQIGGSYFKEPBBVILG |
| ARG_TT | RVGVMVLAHADTNPETSPSGNHICPMLAASLGFGHAPLTQIGGSYFKEPBBVILG |
**Figure S8. A) Sequence alignment of ureohydrolases.** The sequences of eleven ureohydrolases [AMH_A828: amidinohydrolase from *Streptomyces olivaceus* Tü4018 (A828); AMH_A828: amidinohydrolase from *Saccharomonospora caesia* (A821); GbuA_PA: guanidinobutyrase from *Pseudomonas aeruginosa*; GpuA_PA: guanidinopropionase from *Pseudomonas aeruginosa*; PAH_SC: proclavaminic acid amidino hydrolase (PAH) from *Streptomyces clavuligerus*; Agm_BT: agmatinase from *Burkholderia thailandensis*; Agm_DR: agmatinase from *Deinococcus radiodurans*; Agm_CD: agmatinase from *Clostridium difficile*; Agm_TV: agmatinase from *Thermoplasma volcanium*; ARG_BC: Arginase from *Bacillus caldovelox*; ARG_TT: Arginase from *Thermus Thermophilus*] are aligned using MultAlin. Three well-conserved sequences (xGGDH, DAHxD, and SxDxDxxDPxxxP) in most of the ureohydrolases are indicated by black boxes. The metal binding sites are indicated with asterisks, guanidino ligands with black triangles. **B) Cladogram of amidinohydrolases AMH_A828, AMH_A821, and homologues.** Analyses were performed using FigTree v1.4.2.0.

![Image of gel electrophoresis](image)

**Figure S9. In-frame deletion of amidinohydrolase gene dstH in Streptomyces macronensis.** Lane 1: marker; Lane 2 and 3: PCR product from ΔdstH (684 bp) and WT (1,770 bp), respectively.
Figure S10. 4 - 12% Bis-Tris SDS-PAGE analysis of DstH. Lane 1, protein standards; Lane 2, DstH (43 kDa).

Figure S11. HPLC-ESI-MS total ion current traces of in vitro amidinohydrolysis of desertomycin B catalysed by DstH in the presence of various divalent ions. Desertomycin B is efficiently converted to its amino form 1a in the presence of either Co²⁺ or Ni²⁺.
Figure S12. HPLC-ESI-MS total ion current traces of in vitro conversion of primycin A1 catalysed by DstH with various divalent ions. Primycin A1 can be efficiently converted to its amino form under the assay conditions used.
Figure S13. HPLC-ESI-MS total ion current traces of in vitro conversion of kanchanamycin C catalysed by DstH with various divalent ions. Kanchanamycin can be almost completely converted to its amino form under the assay conditions used.
Figure S14. HPLC-ESI-MS total ion current traces of in vitro conversion of azalomycin F4a catalysed by DstH with various divalent ions. Azalomycin F4a can not be converted to its amino form under the assay conditions used. The two peaks are isomers of The two peaks are azalomycin F4a isomers, differing in the attachment site of the malonyl group, either at C23-OH or at C25-OH.[8]

3. Supplementary Tables

Table S1. Bacterial strains used in this study.

| Strain       | Genotype/Characteristics                                                                 | Reference |
|--------------|-----------------------------------------------------------------------------------------|-----------|
| E. coli      |                                                                                        | Invitrogen|
| DH10B        | F mcrA Δ(mrr-hsdRMS-mcrBC), Φ80lacZΔM15, Δ lacX74 recA1 endA1 araD139 Δ (ara leu)7697 galU galK rpsL nupG λ- host for general cloning | Invitrogen|
| BL21(DE3)    | F ompT hsdSde(rB, mB ) gal dcm (λDE3 lysogen) host for protein expression                | [9]       |
| ET12567 (pUZ8002) | (F dam-13::Tn9 dcm-6 hsdM hsdR recF143 zjj-202::Tn10 galK2 galT22 ara14 pacY1 xyl-5 leuB6 thi-1) Donor strain for conjugation between E. coli and Streptomyces |           |
Table S2. Plasmids used in this work.

| Plasmid        | Genotype/Characteristics                                                                 | Reference |
|----------------|-----------------------------------------------------------------------------------------|-----------|
| pYH7           | *E. coli*-Streptomyces shuttle vector                                                    | [4]       |
| pYH7-dstH      | dstH gene disruption construct in which a 1635 bp internal fragment of dstH was deleted in-frame | this work |
| pET28a(+)      | *E. coli* protein expression vector                                                      | Invitrogen|
| pET28a-dstH    | dstH protein expression construct with N-terminal His-tag based on pET28a(+)            | this work |

Table S3. Oligonucleotide primers used in this work.

| Primer          | Nucleotide sequence (5' to 3')                                                                 | Restriction site(s) |
|-----------------|------------------------------------------------------------------------------------------------|---------------------|
| *primers for protein expression* | | |
| dstH-fwd        | TTTTCAATATGAGCGAGACACCCGAGTCCGA                                                            | NdeI                |
| dstH-rev        | AGCTGAAGGCTT TCACTTGAGCGGGAAGCGCA                                                        | HindIII             |
| *primers for dstH gene in-frame deletion* | | |
| dstH-up F       | TGATCAAGGCGAATACCTCATATGTCTCTCGAGGAGCAGCACCAGGAC                                         |                     |
| dstH-up R       | GAAGCGGACCTCCTGCTCATCTCTCTCTGAGGAG                                                       |                     |
| dstH-dn F       | GAGATGAGGAGGAGGAGTCCGCTTCCCACCCGCTAAATGGA                                                |                     |
| dstH-dn R       | CCGCGCGGTCGATCCCCGCATATGGACGAGCTTCAGCAACCGACGAGG                                          |                     |
| *Primers for PCR screening of mutants* | | |
| dstH-CP1        | AGACCACCCACCAACCTCATCGG                                                                    |                     |
| dstH-CP2        | ACGGAGGATGAACTCCAGACGAG                                                                   |                     |
Table S4. Properties of genes within the desertomycin/oasomycin biosynthetic gene cluster of *Streptomyces macronensis* (NRRL B-12566)(A861).

| ORF     | Product size (aa) | % identity/similarity | Species                     | Putative Function | Database entry       |
|---------|------------------|------------------------|-----------------------------|-------------------|----------------------|
| dst6241R | 181              | 72/77                  | *S. clavuligerus*           | unknown           | EFG10431.1.1         |
| dst6242R | 348              | 46/56                  | *Stackebrandtia nassauensis* | LuxR regulator    | ADD43191.1           |
| dst6243  | 161              | 69/79                  | *S. sp. C*                  | secreted protein  | ZP_05506603.1       |
| dst6244  | 344              | 54/68                  | *Saccharopolyspora viridis* |                   | ACU98146.1           |
| dst6245  | 260              | 73/83                  | *S. roseosporus*            | 3-hydroxybutyrate dehydrogenase | EFE73556.1 |
| dst6246  | 455              | 84/92                  | *S. sp. Mg1*                | MFS transporter   | EDX26472.1           |
| dst6247  | 667              | 70/75                  | *S. clavuligerus*           | CdaR regulatory protein | EFG10600.1 |
| dst6248R | 650              | 45/62                  | *S. scabiei*                | ABC transporter   | CBG73917.1           |
| dst6249R | 394              | 72/80                  | *S. hygroscopicus*          | alcohol dehydrogenase | ZP_05518441.1 |
| dst6250  | 251              | 48/60                  | *S. albus*                  | TetR regulator    | ZP_047033876.1      |
| dst6251  | 285              | 68/76                  | *S. avermitilis*            | GCN5 related acetyltransferase | BAC68939.1 |
| dst6252  | 764              | 71/80                  | *S. hygroscopicus*          | glucosidase       | ZP_05512145.1       |
| dst6253  | 638              | 89/94                  | *S. avermitilis*            | ABC transporter   | BAC74929.1           |
| dst6254  | 191              | 55/72                  | *Rhodococcus opacus*        | TetR regulator    | BAH52357.1           |
| dst6255R | 278              | 51/65                  | *S. sp. ACTE*               | 4’-phosphopantetheine transferase | EFB64522.1 |
| dst6256R | 416              | 68/78                  | *S. avermitilis*            | dolichol-P-mannosyl transferase | BAC71004.1 |
| Gene ID | Accession | E-value | Organism | Function | Description |
|---------|------------|---------|----------|----------|-------------|
| dst6257R | 160 | — | — | — | — |
| dst6258 | 939 | 40/53 | *Salinispora tropica* | LuxR regulator | ABP55203.1 |
| dst6259R | 201 | 42/54 | *S. albus* | ABC transporter | EFE832221.1 |
| dst6260 | 542 | 66/77 | *S. hygroscopicus* | arginine oxidase | ZP_05517733.1 |
| dst6261R | 287 | 52/73 | *M. aurantiaca* | ABC transporter | EFA33892.1 |
| dst6262R | 314 | 60/73 | *S. aizunensis* | ABC transporter | AAX98195.1 |
| dst6263 | 5249 | 55/66 | *S. aizunensis* | Type I PKS (DstA1) | AAX98184.1 |
| dst6264 | 3299 | 57/68 | *S. aizunensis* | Type I PKS (DstA2) | AAX98184.1 |
| dst6265 | 5030 | 55/66 | *S. aizunensis* | Type I PKS (DstA3) | AAX98186.1 |
| dst6266 | 4874 | 54/65 | *S. aizunensis* | Type I PKS (DstA4) | AAX98184.1 |
| dst6267 | 5331 | 55/66 | *S. platensis* | Type I PKS (DstA5) | BAH02269.1 |
| dst6268 | 3465 | 57/68 | *S. platensis* | Type I PKS (DstA6) | BAH02269.1 |
| dst6269 | 5450 | 49/63 | *Sorangium cellulosum* | Type I PKS (DstA7) | AAA79984.1 |
| dst6270 | 4403 | 54/65 | *S. aizunensis* | Type I PKS (DstA8) | AAX98191.1 |
| dst6271R | 410 | 72/80 | *S. aureofaciens* | peptidase amidase | ABB05108.1 |
| dst6272 | 483 | 81/88 | *Frankia sp.* | peptide amidase | ABW13525.1 |
| dst6273R | 446 | 46/61 | *S. sp.AA4* | glycosyltransferase | WP_03068347.1 |
| dst6274 | 478 | 61/75 | *S. aizunensis* | acyl-CoA ligase | AAX98201.1 |
| dst6275 | 306 | 52/71 | *S. aizunensis* | acyltransferase | AAX98193.1 |
| dst6276 | 419 | 46/62 | *Saccharopolyspora erythraea* | cytochrome P450 amidino-hydrolase | WP_03068340.1 |
| dst6277 | 372 | 64/77 | *Streptosporangium* | — | ACZ87232.1 |
Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.

Table S5. Properties of genes within the desertomycin/oasomycin biosynthetic cluster of *Streptomyces olivaceus* Tü4018 (A828).

| ORF     | Product size (aa) | % identity/similarity | Species                     | Putative function                        | Database entry     |
|---------|-------------------|------------------------|-----------------------------|------------------------------------------|--------------------|
| dst6278 | 249               | 61/77                  | *S. natalensis*             | oleoyl-ACP hydrolase                     | CAC20922.1         |
| dst6279R| 66                | —                      | —                           | —                                        | —                  |
| dst6280 | 21                | —                      | —                           | —                                        | —                  |
| dst6281 | 96                | —                      | —                           | —                                        | —                  |
| dst6282 | 200               | 78/88                  | *Stackebrandtia nassauensis* | LuxR regulator                           | ADD41656.1         |

Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.
| Gene   | Description          | Accession     |
|--------|----------------------|---------------|
| dst6636R | dst661 | 81/87 | S. aurantiacus | ABC transporter | EPH39610.1 |
| dst6637R | 359 | 99/99 | S. sp. NRRL B-1347 | oxido-reductase | WP_030631560.1 |
| dst6638 | 295 | 97/97 | S. sp. NRRL B-1347 | GCN5 related acetyl-transferase | WP_030631563.1 |
| dst6639R | 760 | 99/99 | S. sp. NRRL B-1347 | glucosidase | WP_030631566.1 |
| dst6640 | 627 | 99/100 | S. sp. NRRL B-1347 | ABC transporter | WP_030631569.1 |
| dst6641R | 198 | 99/100 | S. sp. NRRL B-1347 | deaminase/reductase | WP_030631572.1 |
| dst6642 | 200 | 100/100 | S. sp. NRRL B-1347 | TetR regulator | WP_030631573.1 |
| dst6643R | 233 | 58/69 | S. sp. CNS654 | 4'-phosphopantetheine transferase | WP_032768805.1 |
| dst6644R | 436 | 99/99 | S. sp. NRRL B-1347 | dolichol-P-mannosyl-transferase | WP_030631577.1 |
| dst6645 | 916 | 44/56 | Kutzneria albida | LuxR regulator | AHH94593.1 |
| dst6646R | 224 | 41/58 | Cellulomonas sp. HZM | ABC transporter | WP_029291236.1 |
| dst6647 | 542 | 99/99 | S. sp. NRRL B-1347 | arginine oxidase | WP_030631582.1 |
| dst6648R | 271 | 96/98 | S. sp. | ABC transporter | WP_030631563.1 |
| dst6649R | 314 | 99/99 | S. sp. NRRL B-1347 | ABC transporter | WP_030357193.1 |
| dst6650 | 5091 | 57/68 | S. sp. PRh5 | Type I PKS (DstA1) | EXU66032.1 |
| dst6651 | 3247 | 59/69 | S. violaceusniger | Type I PKS (DstA2) | AEM87325.1 |
| dst6652 | 5018 | 57/68 | S. violaceusniger | Type I PKS (DstA3) | AEM83813.1 |
Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.

Table S6. Properties of genes within the desertomycin/oasomycin biosynthetic cluster of *Streptomyces spectabilis* NRRL B-2494.

| ORF     | Product size (aa) | % identity/similarity | Species          | Putative function                      | Database entry |
|---------|-------------------|------------------------|------------------|----------------------------------------|----------------|
| dst1331R | 1095              | 82/87                  | *S. aurantiacus* | SARP regulatory protein                | EPH43652.1     |
| dst1330R | 121               | 48/60                  | *S. fulvissimus* | SnoaL-like                              | AGK79080.1     |
| dst1329R | 141               | 37/57                  | *A. erythraea*   | ketosteroid isomerase                   | KGI826 24.1    |
| dst1328 | 180  | 73/80   | S. fulvissimus | fatty acid binding protein | AGK81799.1 |
| dst1327 | 161  | 68/79   | S. globisporus | phospho-lipase A2          | CDR08492.1 |
| dst1326 | 274  | 99/99   | S. sp. NRRL B-1347 | trypsin | WP_030681536.1 |
| dst1325 | 143  | 96/97   | S. sp. NRRL B-1347 | glyoxalase | WP_030631539.1 |
| dst1324 | 340  | 93/95   | S. sp. NRRL B-1347 | esterase | WP_030631541.1 |
| dst1323 | 265  | 96/97   | S. sp. NRRL B-1347 | 3-hydroxy-butyrate dehydrogenase | WP_030631543.1 |
| dst1322 | 478  | 95/97   | S. sp. NRRL B-1347 | MFS transporter | WP_030631546.1 |
| dst1321 | 647  | 95/96   | S. sp. NRRL B-1347 | CdaR regulatory protein | WP_030631549.1 |
| dst1320R| 661  | 81/88   | S. aurantiacus | ABC transporter | EPH39610.1 |
| dst1319R| 352  | 94/96   | S. sp. NRRL B-1347 | oxido-reductase | WP_030631560.1 |
| dst1318 | 290  | 82/89   | S. sp. NRRL B-1347 | GCN5 related acetyltransferase | WP_030631563.1 |
| dst1317R| 758  | 90/93   | S. sp. NRRL B-1347 | glucosidase | WP_030631566.1 |
| dst1316  | 627  | 98/99   | S. sp. NRRL B-1347 | ABC transporter | WP_030631569.1 |
| dst1315R | 198  | 93/96   | S. sp. NRRL B-1347 | deaminase/reductase | WP_030631572.1 |
| dst1314  | 200  | 91/96   | S. sp. NRRL B-1347 | TetR regulator | WP_030631573.1 |
| dst1313R | 233  | 57/68   | S. tsukubaensis | 4’-phospho-pantetheine transferase | EIF93070.1 |
| dst1312R | 443  | 93/96   | S. sp. NRRL B-1347 | dolichol-P-mannosyltransferase | WP_030631577.1 |
| dst1311  | 916  | 39/53   | A. orientalis | LuxR regulator | ABM47005.1 |
| protein | start | end  | organism            | gene name       | accession     |
|---------|-------|------|---------------------|-----------------|---------------|
| dst1310R | 221   | 34/52| *Longispora albida* | ABC transporter  | WP_018349120.1 |
| dst1309  | 542   | 94/98| *S. sp. NRRL B-1347* | arginine oxidase | WP_030631582.1 |
| dst1308R | 271   | 94/98| *S. aurantiacus*     | ABC transporter  | EPH42743.1   |
| dst1307R | 314   | 95/97| *S. sp. NRRL B-1347* | ABC transporter  | WP_030631585.1 |
| dst1306  | 5067  | 55/67| *S. violaceusniger*  | Type I PKS (DstA1) | AEM83817.1 |
| dst1305  | 3226  | 59/70| *S. violaceusniger*  | Type I PKS (DstA2) | AEM87325.1 |
| dst1304  | 4990  | 57/68| *S. violaceusniger*  | Type I PKS (DstA3) | AEM83813.1 |
| dst1303  | 4717  | 55/65| *S. zinciresistens*  | Type I PKS (DstA4) | EGX61517.1 |
| dst1302  | 5204  | 55/67| *S. zinciresistens*  | Type I PKS (DstA5) | EGX61515.1 |
| dst1301  | 3394  | 55/67| *S. violaceusniger*  | Type I PKS (DstA6) | AEM83812.1 |
| dst1300  | 5315  | 51/62| *S. griseus*         | Type I PKS (DstA7) | EGE45820.1 |
| dst1299  | 4218  | 56/67| *S. violaceusniger*  | Type I PKS (DstA8) | AEM87318.1 |
| dst1298R | 488   | 46/60| *Amycolatopsis jeunensis* | glycosyl transferase | WP_033289361.1 |
| dst1297  | 458   | 96/97| *S. sp. NRRL B-1347* | acyl-CoA ligase | WP_03068349.1 |
| dst1296  | 316   | 96/97| *S. sp. NRRL B-1347* | acyl transferase | WP_03068347.1 |
| dst1295  | 423   | 89/96| *S. aurantiacus*     | cytochrome P450 | EPH42958.1 |
| dst1294  | 372   | 99/99| *S. sp. NRRL B-1347* | amidino hydrolase | WP_03068343.1 |
| dst1293  | 248   | 95/97| *S. sp. NRRL B-1347* | oleoyl-ACP hydrolase | WP_03068340.1 |
| dst1292  | 225   | —    | —                   | —               | —             |
| dst1291R | 158   | —    | —                   | —               | —             |
| dst1290  | 220   | 99/100| *S. sp. NRRL B-1347* | LuxR regulator | WP_03068954.1 |

Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession
Table S7. Properties of genes within the kanchanamycin biosynthetic cluster of *Streptomyces olivaceus* Tü4018 (A828).

| ORF   | Product size (aa) | % identity/similarity | Species                      | Putative function                           | Database entry          |
|-------|-------------------|------------------------|------------------------------|---------------------------------------------|--------------------------|
| kch7034 | 392               | 99/99                  | *Streptomyces* sp. NRRL B-1347 | MFS transporter                             | WP_030684614.1           |
| kch7035R | 588               | 98/98                  | *Streptomyces* sp. NRRL B-1347 | chitinase                                   | WP_030684612.1           |
| kch7036 | 363               | 92/96                  | *S. zinciresistens*           | cytochrome P450 ferredoxin                  | EGX61513.1               |
| kch7037 | 68                | 97/97                  | *Streptomyces* sp. NRRL B-1347 | Type I PKS (KchA2)                          | EXU62707.1               |
| kch7038 | 5181              | 85/90                  | *S. sp.* PRh5                  | Type I PKS (KchA3)                          | WP_007491078.1           |
| kch7039 | 3378              | 75/82                  | *S. zinciresistens*           | Type I PKS (KchA4)                          | WP_020865963.1           |
| kch7040 | 4721              | 84/89                  | *S. rapamycinicus*            | Type I PKS (KchA5)                          | AEM83815.1               |
| kch7041 | 8229              | 88/93                  | *S. violaceusniger*           | Type I PKS (KchA6)                          | WP_014057314.1           |
| kch7042 | 3170              | 90/94                  | *S. violaceusniger*           | Type I PKS (KchA7)                          | WP_007491082.1           |
| kch7043 | 3460              | 88/91                  | *S. zinciresistens*           | Type I PKS (KchA8)                          | CDR03008.1               |
| kch7044 | 2111              | 87/91                  | *S. iranensis*                | Type I PKS                                 | WP_030683821.1           |
| kch7045 | 2296              | 99/99                  | *S. sp.* NRRL B-1347          | MFS transporter                             | WP_007491082.1           |
| kch7046 | 468               | 99/99                  | *S. sp.* NRRL B-1347          | CoA ligase                                  | WP_030683820.1           |
| kch7047 | 313               | 99/99                  | *S. sp.* NRRL B-1347          | acyl transferase                            | WP_030683822.1           |
| kch7048 | 26                | —                      | —                            | arginine oxidase                            | WP_030683823.1           |
| kch7049 | 559               | 98/99                  | *S. sp.* NRRL B-1347          | —                                           | WP_030683824.1           |
| kch7050R| 241               | 96/98                  | *S. sp.* NRRL B-1347          | 4'-phospho-pantetheine transferase          | WP_007491096.1           |
| kch7051R| 122               | 51/52                  | *S. zinciresistens*           | metallo P-esterase                          | WP_007491096.1           |
Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.

Table S8. Properties of genes within the primycin biosynthetic cluster of *Saccharomonospora azurea* DSM 43044 (A821).

| Product size (aa) | % identity/similarity | Species | Putative function | Database entry |
|------------------|------------------------|---------|-------------------|----------------|
| 144              | 68/76                  | *Saccharomonospora erythraea* | cytosine deaminase | ZP_06563012.1  |
| 477              | 63/78                  | *Kribbella flavida*            | propionyl-CoA carboxylase-β subunit | ADB30540.1 |
| 785, 393         | 48/64, 49/61           | *Ralstonia pickettii*          | penicillin acylase membrane protein | ACD29296.1, EFG05096.1 |
| 110, 262, 510    | —, 70/79, 30/48        | —, *S. sp. AA4*, *Rhodococcus erythropolis* | —, ABC transporter | —, CDR08492.1, BAH02269.1 |
| 421              | 47/64                  | *Saccharopolymerospora erythraea* | cytochrome P450 | CAM05122.1 |

Putative functions of the encoded proteins were deduced from analyses with the BlastP program. The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.
|    |    |    |                      |                      |
|----|----|----|---------------------|---------------------|
| 501| 45/60 | S. sp. AA4 | glycosyl-transferase | ZP_05481000.1 |
| 338| 39/60 | S. aizunensis | 4-guanidino-butanoate: CoA acyl transferase | AAX98193.1 |
| 5140| 51/64 | S. platensis | Type I PKS (PriA1) | BAH02269.1 |
| 763| 30/42 | Saccharopolyspora viridis | glucose dehydrogenase | ACU98636.1 |
| 7771| 54/65 | S. sp. FR-008 | Type I PKS (PriA2) | AAQ82567.1 |
| 5859| 55/65 | S. avermitilis | Type I PKS (PriA3) | NP_821591.1 |
| 7102| 51/64 | S. sp. NRRL 30748 | Type I PKS (PriA4) | ABC87510.1 |
| 6975| 53/64 | S. ambofaciens | Type I PKS (PriA5) | CAJ88175.1 |
| 1789| xx/yy | S. avermitilis | Type I PKS (PriA6) | NP_821590.1 |
| 375| 57/75 | Streptosporangium roseum | amidino-hydrolase | ACZ87232.1 |
| 67| — | — | — | — |
| 331| 65/78 | S. ghanaensis | ABC transporter | EFE68313.1 |
| 269| 61/80 | S. lividans | ABC transporter | EFD68190.1 |
| 379| 52/68 | S. sp. AA4 | 2-component histidine kinase | ZP_05478573.1 |
| 208| 69/83 | S. sp. AA4 | TetR regulator | ZP_05478572.1 |
| 955| 45/58 | Thermomonospora curvata | LuxR regulator | ACY96337.1 |
| 224| — | — | — | — |
| 184| — | — | — | — |
| 216| 95/97 | Saccharomonospora viridis | response regulator | ACU98248.1 |
| 224| 91/95 | Saccharomonospora viridis | ABC transporter | ACU98247.1 |
| Index | Percentage | Species/Microorganism | Protein Function | Accession Number |
|-------|------------|----------------------|------------------|------------------|
| 440   | 84/93      | Saccharomonomonas viridis | signal transduction histidine kinase | ACU98246.1 |
| 259   | 78/84      | Saccharomonomonas viridis | DeoR regulator | ACU98245.1 |
| 338   | 64/74      | Saccharopolymera erythraea | galactose-1-P-uridyltransferase | CAM00105.1 |
| 351   | 84/92      | Saccharomonomonas viridis | trypsin | ACU98243.1 |
| 159   | 91/97      | Saccharomonomonas viridis | molybdo-pterin cofactor synthesis protein | ACU98242.1 |
| 148   | 65/75      | Saccharomonomonas viridis | SAF domain protein | ACU98240.1 |
| 56    |            | Saccharomonomonas viridis | 5-formylTHF carboligase | ACU98238.1 |
| 95    | 75/84      | Saccharomonomonas viridis | UDP-glucose pyrophosphorylase | ACU98237.1 |
| 300   | 90/94      | Saccharomonomonas viridis | molybdo-pterin biosynthetic protein | ACU98236.1 |
| 422   | 93/95      | Saccharomonomonas viridis | membrane protein | BAH53947.1 |
| 225   | 78/84      | Saccharomonomonas viridis | Rhodococcus opacus | ACU98235.1 |
| 290   | 36/48      | Saccharomonomonas viridis | CoA ligase | AAX98201.1 |
| 471   | 62/74      | S. aizunensis | oleoyl-ACP hydrolase | ZP_05520438.1 |
| 202   | 67/80      | S. hygroscopicus | LuxR regulator | ABP55203.1 |
| 173   |            | Salinispora tropica | guanidino-butyramide hydrolase | ZP_05530153.1 |
| 551   | 74/82      | S. hygroscopicus | arginine oxidase | ZP_05517733.1 |
| 447   | 94/97      | Saccharomonomonas viridis | glutamate dehydrogenase | ACU98231.1 |
| 613   | 58/70      | Saccharomonomonas viridis | protein kinase | ACU98230.1 |
| 609   | 60/71      | Saccharomonomonas viridis | tryptophan tRNA synthetase | ACU98228.1 |
| 332   | 90/94      | Saccharomonomonas viridis | acyl-CoA | ACU98226.1 |
| 632   | 83/90      | Saccharomonomonas viridis | | |

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Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.

Table S9. Properties of genes within the azalomycin biosynthetic cluster of *Streptomyces violaceusniger* DSM4137 (A819).

| ORF   | Product size (aa) | % identity/similarity | Species                              | Putative function                      | Database entry       |
|-------|-------------------|------------------------|--------------------------------------|----------------------------------------|----------------------|
| azl7488R | 1552              | 88/92                  | *Streptomyces violaceusniger*        | hypothetical protein                   | WP_014057304.1      |
| azl7489 | 151               | —                      | —                                   | —                                      | WP_044567507.1      |
| azl7490 | 248               | 88/95                  | *S. iranensis*                      | cytochrome P450                         | WP_044567505.1      |
| azl7491 | 68                | 97/97                  | *S. iranensis*                      | ferredoxin Type I PKS (AzlA2)          | EXU62707.1           |
| azl7492 | 5180              | 85/90                  | *S. sp. PRh5*                       | Type I PKS (AzlA3)                     | WP_007491078.1      |
| azl7493 | 3376              | 76/83                  | *S. zinciresistens*                 | Type I PKS                             | WP_020865963.1      |
| azl7494 | 4713              | 84/89                  | *S. rapamycinicus*                  | —                                      | —                   |
| azl7495 | 8259              | 88/93                  | *S. violaceusniger*                 | Type I PKS                             | AEM83815.1           |
| azl7496 | 3166              | 92/95                  | *S. violaceusniger*                 | Type I PKS                             | WP_014057314.1      |
| azl7497 | 3453              | 90/92                  | *S. zinciresistens*                 | Type I PKS                             | WP_007491082.1      |
| azl7498 | 2109              | 87/91                  | *Streptomyces sp. NRRL B-1347*       | Type I PKS                             | WP_037826258.1      |
| azl7499 | 2308              | 94/96                  | *S. sp. NRRL B-1347*                | Type I PKS                             | WP_030683812.1      |
| azl7500 | 478               | 99/99                  | *Streptomyces sp. PRh5*             | CoA ligase                             | WP_037957079.1      |
| azl7501 | 309               | 94/96                  | *Streptomyces sp. PRh5*             | acyl transferase                       | WP_037957076.1      |
| azl7502 | 126               | 94/96                  | *S. violaceusniger*                 | HxIR regulator endoribo-               | WP_014057322.1      |
| azl7503R| 135               | 93/97                  | *S. violaceusniger*                 | —                                      | WP_014057323.1      |
Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The sequence has been deposited under the accession number XXXXX.

Table S10. High-resolution MS analysis of desertomycin A and desertomycin B produced from *Streptomyces macaronensis*.

| Compound name                  | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------|------------------|--------------------|-------------|-----------------------|
| Desertomycin A (major)         | 1192.7546        | 1192.7565          | -1.6        | C_{61}H_{110}NO_{21}  |
| Desertomycin B (minor)         | 1234.7764        | 1234.7783          | -1.5        | C_{62}H_{112}N_{3}O_{21} |

Table S11. High-resolution MS analysis of desertomycin B produced from *dstH*-deletion mutant of *Streptomyces macaronensis*.

| Compound name                  | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------|------------------|--------------------|-------------|-----------------------|
| Desertomycin B                 | 1234.7777        | 1234.7783          | -0.5        | C_{62}H_{112}N_{3}O_{21} |

Table S12. High-resolution MS analysis of desertomycin A and kanchanamycins produced from *Streptomyces olivaceus* Tü4018.

| Compound name                  | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------|------------------|--------------------|-------------|-----------------------|
| Desertomycin A                 | 1192.7548        | 1192.7565          | -1.4        | C_{61}H_{110}NO_{21}  |
| Compound name                        | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------------|------------------|--------------------|-------------|-----------------------|
| Kanchanamycin-1011                   | 1012.6190        | 1012.6203          | -1.3        | C_{53}H_{90}NO_{17}   |
| Kanchanamycin-1053                   | 1054.6408        | 1054.6421          | -1.2        | C_{54}H_{92}N_{17}O_{17} |
| Kanchanamycin-925                    | 926.6190         | 926.6199           | -1.0        | C_{50}H_{88}NO_{14}   |
| Kanchanamycin-967                    | 968.6405         | 968.6417           | -1.2        | C_{51}H_{90}N_{17}O_{14} |

Table S13. High-resolution MS analysis of primycins produced from *Saccharomonospora azurea* DSM 43044.

| Compound name                        | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------------|------------------|--------------------|-------------|-----------------------|
| Primycin-1035                        | 1036.7134        | 1036.7142          | -0.8        | C_{54}H_{102}NO_{17}  |
| Primycin-1049                        | 1050.7290        | 1050.7299          | -0.9        | C_{55}H_{104}NO_{17}  |
| Primycin-1063                        | 1064.7444        | 1064.7455          | -1.0        | C_{56}H_{106}NO_{17}  |
| Primycin-1077 (Primycin A1)          | 1078.7354        | 1078.7360          | -0.6        | C_{56}H_{104}N_{3}O_{17} |
| Primycin-1091 (Primycin A2)          | 1092.7505        | 1092.7517          | -1.1        | C_{56}H_{106}N_{3}O_{17} |
| Primycin-1105 (Primycin A3)          | 1106.7660        | 1106.7673          | -1.2        | C_{57}H_{108}N_{3}O_{17} |

Table S14. High-resolution MS analysis of azalomycin F4a produced from *Streptomyces violaceusniger* DSM4137.

| Compound name                        | Observed [M+H]^+ | Calculated [M+H]^+ | Error (ppm) | Formula               |
|--------------------------------------|------------------|--------------------|-------------|-----------------------|
| Azalomycin F4a                       | 1082.6722        | 1082.6734          | -1.1        | C_{56}H_{90}N_{3}O_{17} |

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