# Supporting Information

## Claesen and Bibb 10.1073/pnas.1008608107

### SI Materials and Methods

**PCR Targeting.** PCR targeting primer couples (*orf1* F/*orf1* R) to (*cypI* F/*cypI* R) were used to amplify an apramycin resistance cassette from pIJ773 that also contains an *oriT* and is flanked by Flippase recombination enzyme (FLP) recognition sites. The resulting cassettes were used to delete *orf1* to *cypI* in pIJ12404, resulting in cosmids pIJ12422–pIJ12430. These constructs were introduced into *Streptomyces* sp. OH-4156 by conjugation and selecting for apramycin resistance. Screening the apramycin-resistant exconjugants for loss of kanamycin resistance (encoded by *neo* of SuperCosI) identified double cross-over mutants M1427–M1435 that each have a single wild-type ORF (*orf1* to *cypI*) replaced by the pIJ773-derived cassette. The mutants were confirmed by PCR analysis using the corresponding primer couples (*orf1* T1/*orf1* T2) to (*cypI* T1/*cypI* T2). To avoid any polar effects of the mutations on the expression of downstream genes, we also constructed in-frame (scar) deletion mutations for all the putative *cyp* genes (*orf1* to *cypI*). These mutations were made by introducing pIJ12422–pIJ12430 into *Escherichia coli* BT340 and growth at 42 °C. This procedure resulted in the FLP-mediated excision of the apramycin resistance cassette, leaving an 81-bp in-frame 'scar' sequence (1) and yielding pIJ12431–pIJ12439 (Δ*orf1*–Δ*cypI*). Derivatives capable of integrating into the chromosome of a *Streptomyces* host were made by inserting a 5.2-kb SspI fragment from pIJ10702 that contained *oriT*, the apramycin resistance gene [*aac(3)IV*], and the ϕC31 *attP* site and integrase gene by PCR targeting. The resulting cosmids (pIJ12440–pIJ12448) were transferred to *S. coelicolor* M1146, where stable integration at the ϕC31 *attB* site occurred; no antibiotic selection was required to maintain the cosmids after integration. Each of the resulting strains (M1413–M1421) contained a cosmid with an individual gene (Δ*orf1*–Δ*cypI*, respectively) of the putative minimal gene set replaced by a scar sequence.

Similarly, *cypA*-containing cosmids from the *S. sp.* OH-4156 genomic library identified by hybridization (pIJ12400–pIJ12408) were targeted with the 5.2-kb pIJ10702 SspI fragment to yield integrative versions (pIJ12409–pIJ12417, respectively). These cosmids then were introduced into *Streptomyces venezuelae* ATCC 10712 by conjugation, yielding strains M1400–M1409.

**Construction of Fusion Proteins.** Gateway primer couples (Gat *cypA* F/Gat *cypA* R, Gat *cypD* F/Gat *cypD* R, and Gat *cypM* F/Gat *cypM* R) were used to amplify *cypA*, *cypD*, and *cypM*, respectively. The resulting PCR products were introduced into the pDONR207 entry vector and shuttled into the pHM-GWA destination vector following the protocol of the Gateway cloning kit (Invitrogen). The resulting plasmids pIJ12489 (*cypA*), pIJ12493 (*cypD*), and pIJ12494 (*cypM*) were introduced into *E. coli* BL21 (DE3) by transformation. Recombinant 6-His–tagged maltose-binding protein (MBP)-CypA, 6-His–tagged MBP-CypD, and 6-His–tagged MBP-CypM were purified from 3 L of Lennox medium [L medium; tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L (2)] after induction with 0.5 mM isopropyl-β-D-1-thiogalactopyranoside at 30 °C for 4 h. Cell pellets were resuspended in 40 mL lysis buffer (50 mM Tris HCl, pH 7.9, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride), lysed with a French press, and the cell lysate was clarified by centrifugation (20 min at 40,000 × *g*). The cleared lysate was applied to a 1-mL $Ni2^{+}$-loaded Hi-Trap Chelating HD column (GE Healthcare), washed with 25 mM $K_2HPO_4$ (pH 7.9), 200 mM NaCl, and 20 mM imidazole, and eluted with an imidazole gradient. The eluted proteins were dialyzed into storage buffer [50 mM Tris HCl (pH 7.9), 50 mM NaCl, 1 mM Tris(2-carboxyethyl) phosphine (TCEP)], aliquoted, and stored at −80 °C. Protein concentrations were determined by a Bradford assay according to the manufacturer's instructions (Bio-Rad). BSA was used as the protein standard. We obtained preproCypA by digestion of 6-His–tagged MBP-CypA with tobacco etch virus protease following the Invitrogen protocol.

**CypD Assay.** We incubated 6-His–tagged MBP-CypD (~25 μM) with the preproCypA substrate (~120 μM) at 30 °C in a 20-μL volume at pH 7.9 (50 mM Tris HCl) under reducing conditions (2 mM TCEP). After 6 h incubation, the reaction mixture was analyzed by MALDI-TOF MS. The substrate peak with a mass of 6,087 Da was partially converted into a peak of 6,041 Da (Fig. S7 *A* and *B*). The 46-Da difference in mass is in good agreement with the removal of two hydrogen atoms and one molecule of $CO_2$.

**CypM Assay.** We incubated 6-His–tagged MBP-CypM (~7.5 μM) with nonmethylated cypemycin (unknown concentration) at 30 °C in a 20-μL volume at pH 7.9 (50 mM Tris HCl) under reducing conditions (2 mM TCEP) in the presence of added *S*-adenosyl methionine (2.5 mM) and $MgCl_2$ (10 mM). After 3 h incubation, the reaction mixture was analyzed by MALDI-TOF MS. The peaks corresponding to the nonmethylated substrate ($[M+H]^+$ = 2,068 Da; $[M+Na]^+$ = 2,090 Da; $[M+K]^+$ = 2,106 Da) were mostly converted into mature cypemycin ($[M+Na]^+$ = 2,118 Da; $[M+K]^+$ = 2,134 Da) by the addition of CypM (Fig. S7 *C* and *D*).

1. Gust B, et al. (2004) Lambda Red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. *Adv Appl Microbiol* 54:107–128.
2. Lennox ES (1955) Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* 1:190–206.

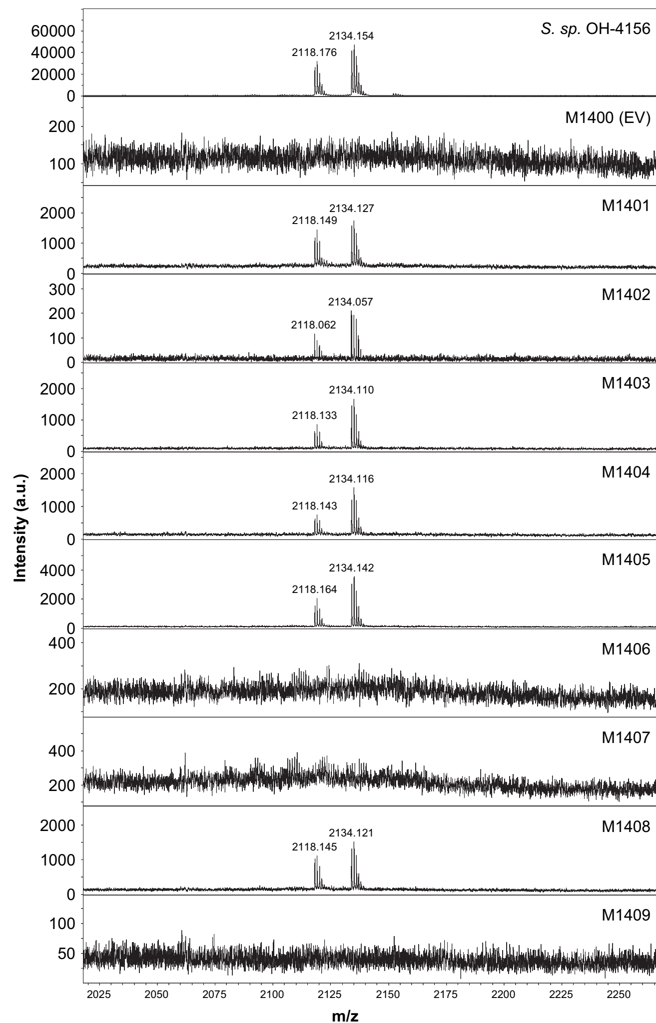



**Fig. S1.** Heterologous expression of the cypemycin biosynthetic gene cluster in *Streptomyces venezuelae.* MALDI-TOF spectra for *Streptomyces* sp. OH-4156, *S. venezuelae* with the empty vector pIJ10702 (M1400), and *S. venezuelae* derivatives with cosmids containing all the genes required for cypemycin production (M1401, M1402, M1403, M1404, M1405, and M1408) and those with a cosmid that does not (M1406, M1407, and M1409).

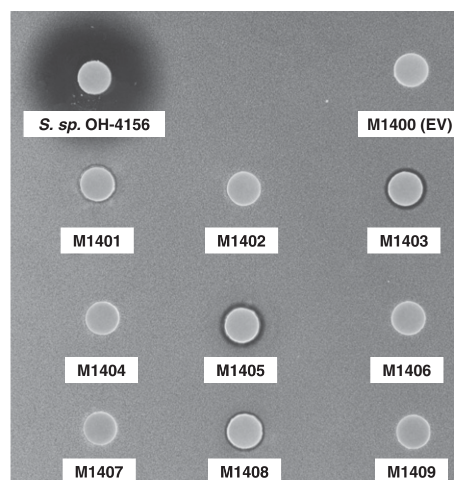



**Fig. S2.** *Micrococcus luteus* bioassay of the nine *S. venezuelae* derivatives with a *cypA*-containing cosmid. *Streptomyces* sp. OH-4156 (positive control), M1400 (empty vector, EV; negative control), and M1401–M1409 are the *S. venezuelae* clones containing hybridizing library cosmids. The halos observed for M1401, M1403, M1405, and M1408 are much smaller than that of the natural producer; although clones M1402 and M1404 failed to produce a halo, cypemycin production was confirmed by MALDI-TOF analysis.

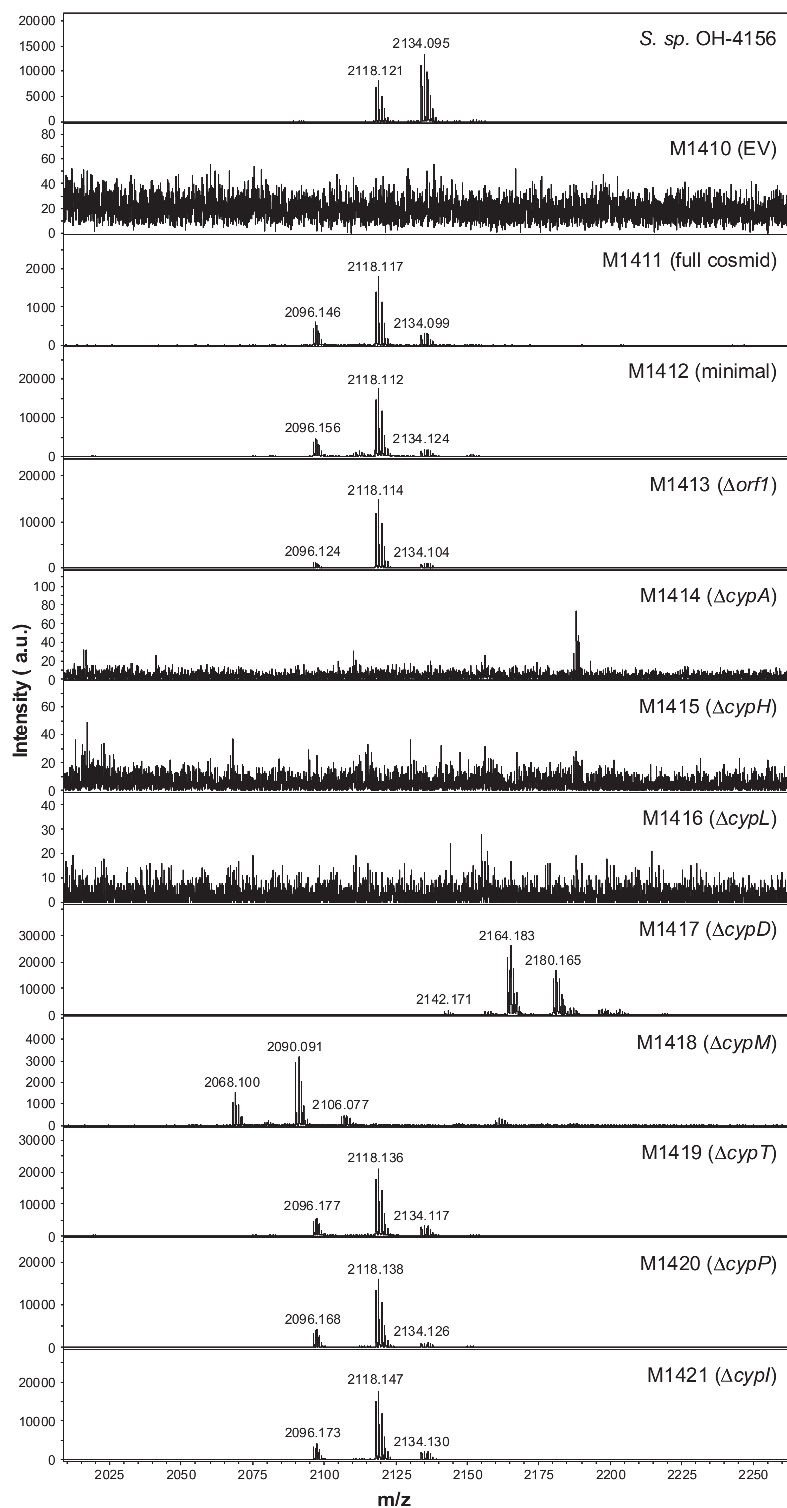



**Fig. S3.** MALDI-TOF spectra for scar mutants in M1146. Spectra for *Streptomyces* sp. OH-4156 (positive control), M1146 with the inserted empty vector pIJ10702 (M1410), entire cosmid (M1411), minimal gene set (M1412), and individual mutants in *orf1* to *cypI* (M1413–M1421). Cypemycin is detected as $[M+H]^+$ = 2,096 Da, $[M+Na]^+$ = 2,118 Da, and $[M+K]^+$ = 2,134 Da. The nondecarboxylated version of cypemycin (in M1417) appears as $[M+H]^+$ = 2,142 Da, $[M+Na]^+$ = 2,164 Da, and $[M+K]^+$ = 2,180 Da, and the nonmethylated intermediate (in M1418) as $[M+H]^+$ = 2,068 Da, $[M+Na]^+$ = 2,090 Da, and $[M+K]^+$ = 2,106 Da.

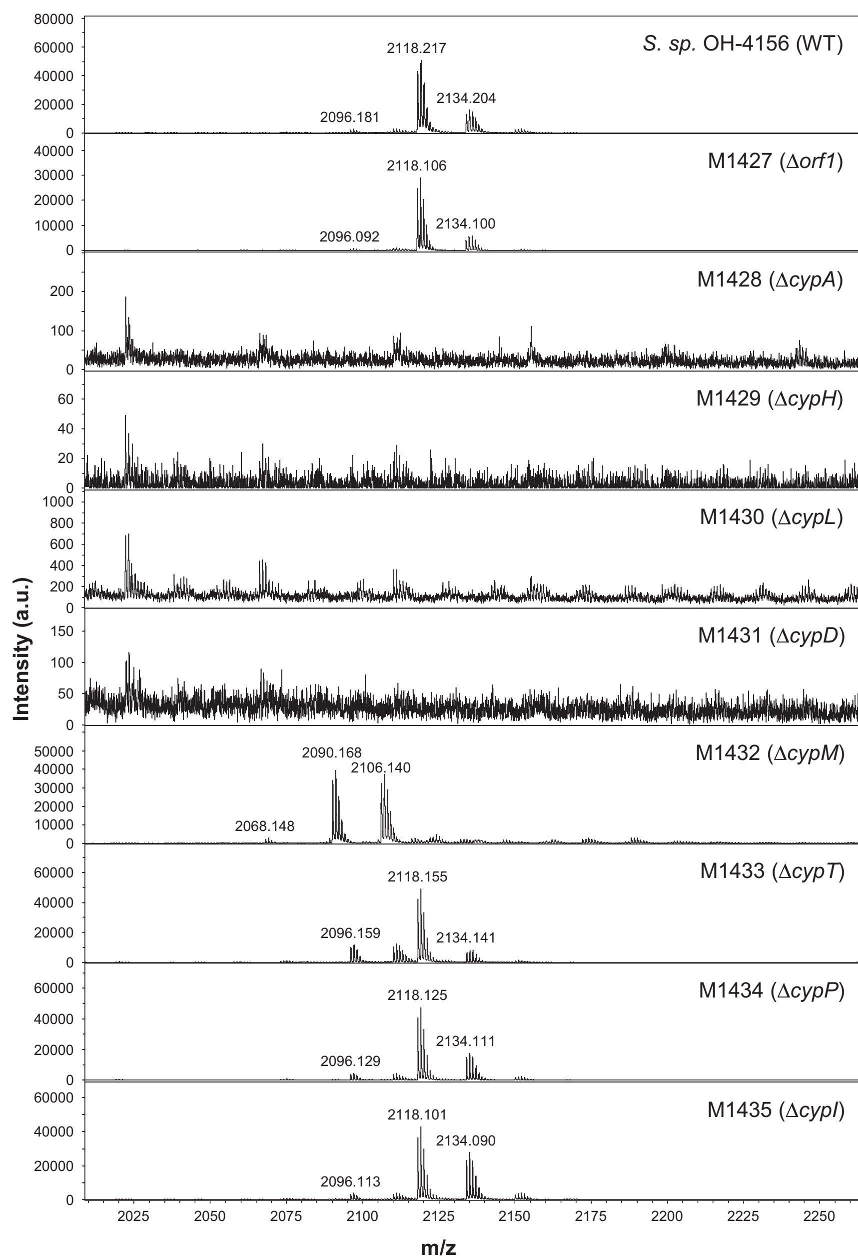
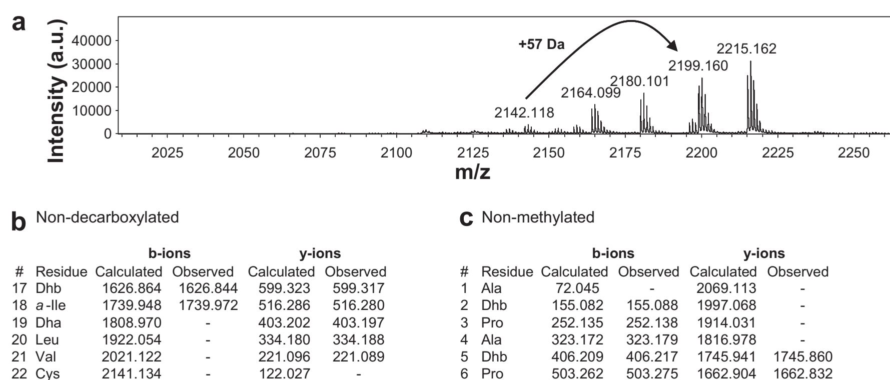



**Fig. S4.** MALDI-TOF spectra of the apramycin-marked mutants in *Streptomyces* sp. OH-4156. Spectra for M1427 (Δ*orf1*) to M1435 (Δ*cypI*) show the same phenotypes as the heterologously expressed mutants (Fig. S5). No cypemycin intermediate was detected in the culture of M1431 (Δ*cypD*), whereas its heterologous counterpart (M1417) produced nondecarboxylated cypemycin. A possible explanation is that the replacement of *cypD* in this mutant with the pIJ773-derived cassette causes a polar effect on the downstream *cypM* gene, making M1431 a Δ*cypDM* double mutant. The product of this mutant, cypemycin lacking the *S*-[(Z)-2-aminovinyl]-D-cysteine (AviCys) and *N*,*N*-dimethylalanine ($Me_2$-Ala), is thought to be unstable but has been isolated from a heterologously expressing Δ*cypDM* double mutant. Differences in protease activity between M1146 and *Streptomyces* sp. OH-4156 could account for this observation.

**Fig. S5.** Quadrupole TOF (Q-TOF) spectrum of cypemycin. Fragmentation of the modified linear peptide allows determination of the amino acid sequence and the nature and locations of modified residues. The amino acid sequence between Pro3 and the AviCys residue (which does not fragment easily into interpretable masses) can be readily discerned and is shown above the spectrum.




**b** Non-decarboxylated

| # | Residue | b-ions Calculated | b-ions Observed | y-ions Calculated | y-ions Observed |
|---|---|---|---|---|---|
| 17 | Dhb | 1626.864 | 1626.844 | 599.323 | 599.317 |
| 18 | *a*-Ile | 1739.948 | 1739.972 | 516.286 | 516.280 |
| 19 | Dha | 1808.970 | - | 403.202 | 403.197 |
| 20 | Leu | 1922.054 | - | 334.180 | 334.188 |
| 21 | Val | 2021.122 | - | 221.096 | 221.089 |
| 22 | Cys | 2141.134 | - | 122.027 | - |

**c** Non-methylated

| # | Residue | b-ions Calculated | b-ions Observed | y-ions Calculated | y-ions Observed |
|---|---|---|---|---|---|
| 1 | Ala | 72.045 | - | 2069.113 | - |
| 2 | Dhb | 155.082 | 155.088 | 1997.068 | - |
| 3 | Pro | 252.135 | 252.138 | 1914.031 | - |
| 4 | Ala | 323.172 | 323.179 | 1816.978 | - |
| 5 | Dhb | 406.209 | 406.217 | 1745.941 | 1745.860 |
| 6 | Pro | 503.262 | 503.275 | 1662.904 | 1662.832 |

**Fig. S6.** Structural verification of the truncated cypemycin derivatives. (*A*) After alkylation of the free thiol groups in the product of the Δ*cypD* mutant, a single mass shift of +57 Da was observed indicating the presence of only one free thiol group. Nondecarboxylated cypemycin appears as $[M+H]^+$ = 2,142 Da, $[M+Na]^+$ = 2,164 Da, and $[M+K]^+$ = 2,180 Da. The alkylated version is observed as $[M+H]^+$ = 2,199 Da and $[M+O]^+$ = 2,215 Da. (*B*) *Q*-TOF analysis of the compound produced by the Δ*cypD* mutant shows that Cys19 has been dehydrated to dehydroalanine. (*C*) Q-TOF analysis confirming that the mass difference of the compound produced by the Δ*cypM* mutant is localized to the first two amino acids and thus is very likely to reflect the lack of the two N-terminal methyl groups.

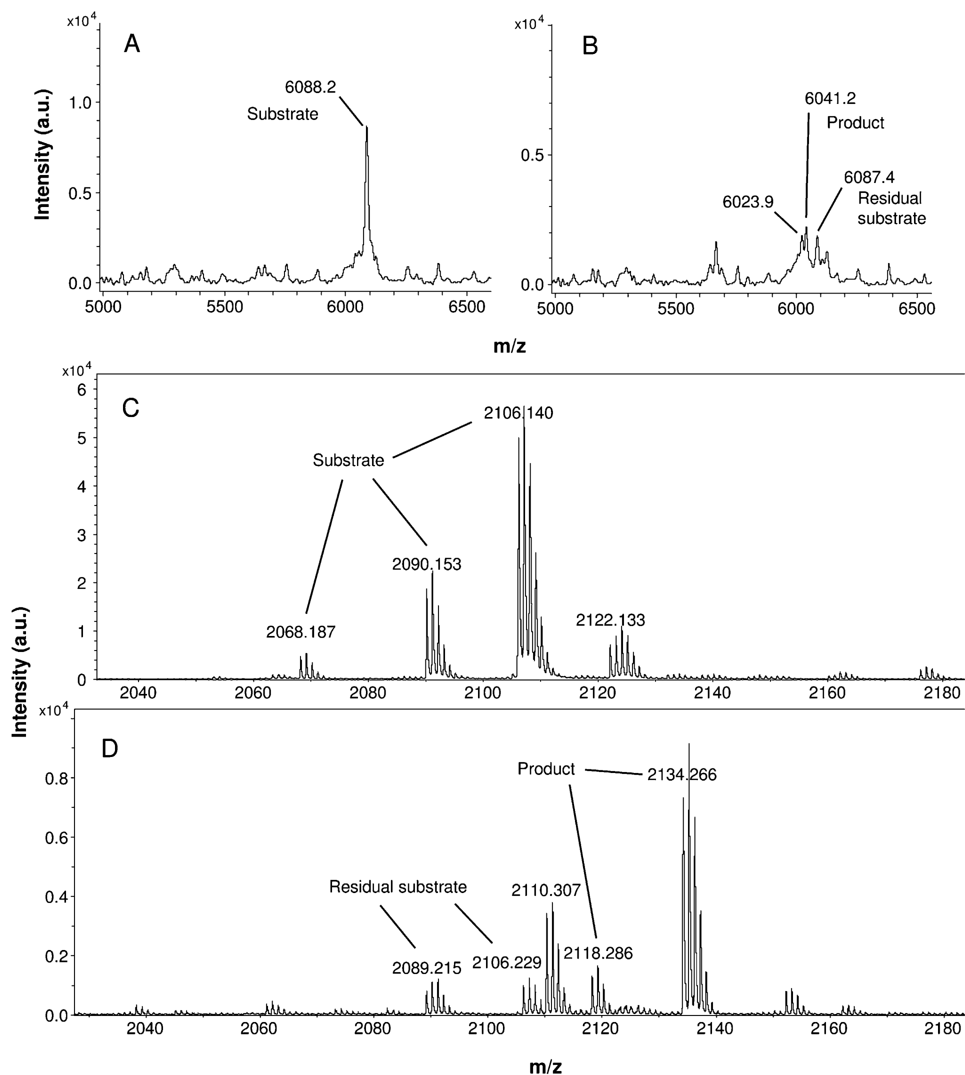



**Fig. S7.** Biochemical assays for CypD decarboxylase and CypM methyltransferase activity. (*A*) Decarboxylation assay control without added CypD. (*B*) Upon incubation with the CypD enzyme, the mass peak corresponding to the 6,087-Da preproCypA substrate is partially converted to the 6,041-Da decarboxylated product. (*C*) Methyl transfer assay control without added CypM. (*D*) Nonmethylated cypemycin ($[M+H]^+$ = 2,068 Da, $[M+Na]^+$ = 2,090 Da, and $[M+K]^+$ = 2,106 Da) was converted to mature cypemycin ($[M+Na]^+$ = 2,118 Da and $[M+K]^+$ = 2,134 Da) upon incubation with CypM.

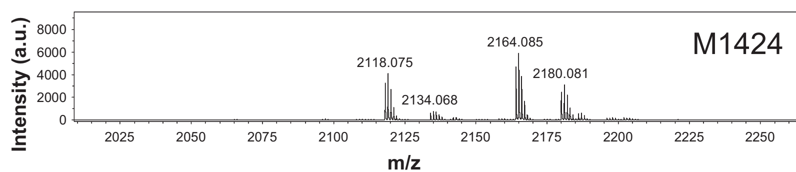



**Fig. S8.** Analysis of the complementation of the Δ*cypL* mutant M1424 confirms production of both cypemycin and the nondecarboxylated form.

**Table S1. Plasmids, strains, and primers used in this study**

| Plasmid* | Description | Ref. or source |
|---|---|---|
| General plasmids | | |
| pIJ10257 | *oriT*, ϕBT1 *int-attB*, *hyg*, *ermE*p* | (1) |
| pIJ10702 | *bla*, *aac(3)IV*, *oriT*, ϕC31 *int-attP* (also known as pMJCos1) | (2) |
| pIJ773 | pBS SK+ containing cassette P1-FRT-*oriT-aac(3)IV*-FRT-P2 | (3) |
| pIJ790 | λ-RED (*gam*, *bet*, *exo*), *cat*, *araC*, *rep101*$^{ts}$ | (3) |
| pSET152 | *lacZ*α, pUC19*ori*, RP4*oriT*, ϕC31 *int-attP*, *aac(3)IV* | (4) |
| pUZ8002 | *tra*, *neo*, RP4 | (5) |
| SuperCosI | *neo*, *bla* | Stratagene |
| pDONR 207 | donor vector for Gateway | Invitrogen |
| pHM-GWA | Gateway destination vector for MBP fusions | (6) |
| Library cosmids for heterologous expression in *S. venezuelae* | | |
| pIJ12400 | genomic library cosmid 1N16 in SupercosI | This work |
| pIJ12401 | genomic library cosmid 3O03 in SupercosI | This work |
| pIJ12402 | genomic library cosmid 4J11 in SupercosI | This work |
| pIJ12403 | genomic library cosmid 6C03 in SupercosI | This work |
| pIJ12404 | genomic library cosmid 6I23 in SupercosI | This work |
| pIJ12405 | genomic library cosmid 7P24 in SupercosI | This work |
| pIJ12406 | genomic library cosmid 8B19 in SupercosI | This work |
| pIJ12407 | genomic library cosmid 8G13 in SupercosI | This work |
| pIJ12408 | genomic library cosmid 8J18 in SupercosI | This work |
| pIJ12409 | pIJ12400 with pIJ10702 backbone | This work |
| pIJ12410 | pIJ12401 with pIJ10702 backbone | This work |
| pIJ12411 | pIJ12402 with pIJ10702 backbone | This work |
| pIJ12412 | pIJ12403 with pIJ10702 backbone | This work |
| pIJ12413 | pIJ12404 with pIJ10702 backbone | This work |
| pIJ12414 | pIJ12405 with pIJ10702 backbone | This work |
| pIJ12415 | pIJ12406 with pIJ10702 backbone | This work |
| pIJ12416 | pIJ12407 with pIJ10702 backbone | This work |
| pIJ12417 | pIJ12408 with pIJ10702 backbone | This work |
| Minimal gene set constructs | | |
| pIJ12418 | pIJ12404 targeted with 773 cassette upstream of *orf1* | This work |
| pIJ12419 | pIJ12418 with in-frame deletion upstream of *orf1* | This work |
| pIJ12420 | pIJ12419 targeted with 773 cassette downstream of *cypI* | This work |
| pIJ12421 | pSET152 with minimal gene set excised from pIJ12420 | This work |
| Gene-deletion constructs | | |
| pIJ12422 | pIJ12404 Δ*orf1*::(*oriT-aac(3)IV*) | This work |
| pIJ12423 | pIJ12404 Δ*cypA*::(*oriT-aac(3)IV*) | This work |
| pIJ12424 | pIJ12404 Δ*cypH*::(*oriT-aac(3)IV*) | This work |
| pIJ12425 | pIJ12404 Δ*cypL*::(*oriT-aac(3)IV*) | This work |
| pIJ12426 | pIJ12404 Δ*cypD*::(*oriT-aac(3)IV*) | This work |
| pIJ12427 | pIJ12404 Δ*cypM*::(*oriT-aac(3)IV*) | This work |
| pIJ12428 | pIJ12404 Δ*cypT*::(*oriT-aac(3)IV*) | This work |
| pIJ12429 | pIJ12404 Δ*cypP*::(*oriT-aac(3)IV*) | This work |
| pIJ12430 | pIJ12404 Δ*cypI*::(*oriT-aac(3)IV*) | This work |
| pIJ12431 | pIJ12422 in-frame deletion of *orf1* | This work |
| pIJ12432 | pIJ12423 in-frame deletion of *cypA* | This work |
| pIJ12433 | pIJ12424 in-frame deletion of *cypH* | This work |
| pIJ12434 | pIJ12425 in-frame deletion of *cypL* | This work |
| pIJ12435 | pIJ12426 in-frame deletion of *cypD* | This work |
| pIJ12436 | pIJ12427 in-frame deletion of *cypM* | This work |
| pIJ12437 | pIJ12428 in-frame deletion of *cypT* | This work |
| pIJ12438 | pIJ12429 in-frame deletion of *cypP* | This work |
| pIJ12439 | pIJ12430 in-frame deletion of *cypI* | This work |
| pIJ12440 | pIJ12431 with pIJ10702 backbone | This work |
| pIJ12441 | pIJ12432 with pIJ10702 backbone | This work |
| pIJ12442 | pIJ12433 with pIJ10702 backbone | This work |
| pIJ12443 | pIJ12434 with pIJ10702 backbone | This work |
| pIJ12444 | pIJ12435 with pIJ10702 backbone | This work |
| pIJ12445 | pIJ12436 with pIJ10702 backbone | This work |
| pIJ12446 | pIJ12437 with pIJ10702 backbone | This work |
| pIJ12447 | pIJ12438 with pIJ10702 backbone | This work |
| pIJ12448 | pIJ12439 with pIJ10702 backbone | This work |
| Complementation constructs | | |
| pIJ12449 | pIJ10257 containing cloned *cypA* | This work |

**Table S1. Cont.**

| Plasmid* | Description | Ref. or source |
|---|---|---|
| pIJ12450 | pIJ10257 containing cloned *cypH* | This work |
| pIJ12451 | pIJ10257 containing cloned *cypL* | This work |
| pIJ12452 | pIJ10257 containing cloned *cypD* | This work |
| pIJ12453 | pIJ10257 containing cloned *cypM* | This work |
| Fusion protein constructs | | |
| pIJ12489 | pHM-GWA containing cloned *cypA* | This work |
| pIJ12493 | pHM-GWA containing cloned *cypD* | This work |
| pIJ12494 | pHM-GWA containing cloned *cypM* | this work |

| Strain | Description | Ref. |
|---|---|---|
| *Micrococcus luteus* | | |
| ATCC4698 | Indicator strain in bio-assay | (2) |
| *Escherichia coli* | | |
| BT340 | DH5α/pCP20 | (7) |
| BW25113 | K-12 derivative: Δ*araBAD*, Δ*rhaBAD* | (8) |
| DH5α | strain used for general cloning and plasmid maintenance | Stratagene |
| ET12567 | *dam, dcm, hsdM, hsdS, hsdR, cat, tet* | (9) |
| XL1-Blue | strain used for SupercosI library | Stratagene |
| BL21 (DE3) | strain used for fusion protein production | (10) |
| *Streptomyces venezuelae* | | |
| ATCC 10712 | Wild type strain; used to construct the *S. venezuelae* derivatives | (11) |
| M1400 | pIJ10702 in ϕC31 *attB* (empty vector) | This work |
| M1401 | pIJ12409 in ϕC31 *attB* | This work |
| M1402 | pIJ12410 in ϕC31 *attB* | This work |
| M1403 | pIJ12411 in ϕC31 *attB* | This work |
| M1404 | pIJ12412 in ϕC31 *attB* | This work |
| M1405 | pIJ12413 in ϕC31 *attB* | This work |
| M1406 | pIJ12414 in ϕC31 *attB* | This work |
| M1407 | pIJ12415 in ϕC31 *attB* | This work |
| M1408 | pIJ12416 in ϕC31 *attB* | This work |
| M1409 | pIJ12417 in ϕC31 *attB* | This work |
| *Streptomyces coelicolor* | | |
| M1146 | M145 Δ*act* Δ*red* Δ*cpk* Δ*cda*; parental strain used to construct the *S. coelicolor* derivatives | J. P. Gomez-Escribano |
| M1410 | pIJ10702 in ϕC31 *attB* (empty vector) | This work |
| M1411 | pIJ12413 in ϕC31 *attB* (full cosmid insert) | This work |
| M1412 | pIJ12421 in ϕC31 *attB* (minimal gene set) | This work |
| M1413 | pIJ12440 in ϕC31 *attB* (Δ*orf1*) | This work |
| M1414 | pIJ12441 in ϕC31 *attB* (Δ*cypA*) | This work |
| M1415 | pIJ12442 in ϕC31 *attB* (Δ*cypH*) | This work |
| M1416 | pIJ12443 in ϕC31 *attB* (Δ*cypL*) | This work |
| M1417 | pIJ12444 in ϕC31 *attB* (Δ*cypD*) | This work |
| M1418 | pIJ12445 in ϕC31 *attB* (Δ*cypM*) | This work |
| M1419 | pIJ12446 in ϕC31 *attB* (Δ*cypT*) | This work |
| M1420 | pIJ12447 in ϕC31 *attB* (Δ*cypP*) | This work |
| M1421 | pIJ12448 in ϕC31 *attB* (Δ*cypI*) | This work |
| M1422 | pIJ12441 in ϕC31 *attB* (Δ*cypA*) and pIJ12449 in ϕBT1 *attB* | This work |
| M1423 | pIJ12442 in ϕC31 *attB* (Δ*cypH*) and pIJ12450 in ϕBT1 *attB* | This work |
| M1424 | pIJ12443 in ϕC31 *attB* (Δ*cypL*) and pIJ12451 in ϕBT1 *attB* | This work |
| M1425 | pIJ12444 in ϕC31 *attB* (Δ*cypD*) and pIJ12452 in ϕBT1 *attB* | This work |
| M1426 | pIJ12445 in ϕC31 *attB* (Δ*cypM*) and pIJ12453 in ϕBT1 *attB* | This work |
| *Streptomyces* sp. OH-4156 | | |
| WT | Wild type strain; used to construct the *S. sp.* OH-4156 mutants | (12) |
| M1427 | Δ*orf1*::(*oriT-aac(3)IV*) | This work |
| M1428 | Δ*cypA*::(*oriT-aac(3)IV*) | This work |
| M1429 | Δ*cypH*::(*oriT-aac(3)IV*) | This work |
| M1430 | Δ*cypL*::(*oriT-aac(3)IV*) | This work |
| M1431 | Δ*cypD*::(*oriT-aac(3)IV*) | This work |
| M1432 | Δ*cypM*::(*oriT-aac(3)IV*) | This work |
| M1433 | Δ*cypT*::(*oriT-aac(3)IV*) | This work |
| M1434 | Δ*cypP*::(*oriT-aac(3)IV*) | This work |
| M1435 | Δ*cypI*::(*oriT-aac(3)IV*) | This work |

**Table S1. Cont.**

| Strain | Description | Ref. |
|---|---|---|
| Primer | Sequence | Site |
| Degenerate primers and probes for *cypA* | | |
| Cyp deg F | gcnacnccngcnacnc | |
| Cyp deg R | rcanacnawdagngtn | |
| Cyp probe 1 | gcsacsccsgcsacsccsacsgtsgcscagttcgtsatccaggs | |
| Cyp probe 2 | gcsacsccsgcsacsccsacsgtsgcscagttcgtsatccagggstcsacsatctcsctsgtstgc | |
| *cypA*-specific probe/test primers | | |
| *cypA* T1 | gacggtctcttgaaggtctg | |
| *cypA* T2 | cctccgccacttccatc | |
| Minimal gene set PCR-targeting primers and test primers | | |
| Up F | agaattcgcggccgcataatacgactcactatagggatcattccggggatccgtcgacc | |
| Up R | gccccgtaccgctggggcacgggcccgggggcgggtcggtctagatgtaggctggagctgcttc | XbaI |
| Down F | ggaggggcggcggggccgcgccgtgccgggcgtcgtcgcaatattattccggggatccgtcgacc | SspI |
| Down R | caagaattcgcggccgcaattaaccctcactaaaggatctgtaggctggagctgcttc | |
| Up T1 | tggaatgaacaatggaagtcaac | |
| Up T2 | cggtgaagtacgggc | |
| Down T1 | gagttcccgctgctgag | |
| Down T2 | ccgaaaagtgccacctgac | |
| Individual gene-deletion PCR-targeting primers and test primers | | |
| *orf1* F | caccgcgtccggggggtgccgtcatggaacggcccggctaactagtattccggggatccgtcgacc | SpeI |
| *orf1* R | gacgggatgggcgggcacgcgcggaggggggacgcacatggctagctgtaggctggagctgcttc | NheI |
| *cypA* F | gaccaccgacgaagggttagtgaagtgcgatctgagatgactagtattccggggatccgtcgacc | SpeI |
| *cypA* R | gtgacgggcctctccggcggcaggctcagtcccgcgtcagctagctgtaggctggagctgcttc | NheI |
| *cypH* F | tctacctgctgccgcgcgagcaggcctcgtacgcgctccactagtattccggggatccgtcgacc | SpeI |
| *cypH* R | gatcgaccggtgcgccgagccgcgcaccacctcgaacttgctagctgtaggctggagctgcttc | NheI |
| *cypL* F | ggggcgctcgccgcgcacctggtcgcgaccgtgctgcagactagtattccggggatccgtcgacc | SpeI |
| *cypL* R | cactcggaggtgaggatcggcttcatccgctggtccgcggctagctgtaggctggagctgcttc | NheI |
| *cypD* F | ccatgtcacgggctccatcagcgcggcgctcgtgccgtgactagtattccggggatccgtcgacc | SpeI |
| *cypD* R | ggcaggttgaaccccacctcggccgtctgccggttgctcgctagctgtaggctggagctgcttc | NheI |
| *cypM* F | gccaacaggatgcggaaggaagggcgttccggtgagtgaactagtattccggggatccgtcgacc | SpeI |
| *cypM* R | tggcgccctccgcggcgatcgccggcacttcgctcactggctagctgtaggctggagctgcttc | NheI |
| *cypT* F | cgctcgcgggagatgctggtcgttctggagcggcagtgaactagtattccggggatccgtcgacc | SpeI |
| *cypT* R | ggtcggcctcgaccgcggtgatgctgtccgtggtcatccgctagctgtaggctggagctgcttc | NheI |
| *cypP* F | atggtggacgcctgcgacggcctcatggagctgggatgaactagtattccggggatccgtcgacc | SpeI |
| *cypP* R | cccaggcggaccgggggccactcccgcggggctccgttagctagctgtaggctggagctgcttc | NheI |
| *cypI* F | caggggtcccgcgcgactccgggacggcagggcaggatgactagtattccggggatccgtcgacc | SpeI |
| *cypI* R | cggaggggtcgcggggccgtccccgttccggaggcctcagctagctgtaggctggagctgcttc | NheI |
| *orf1* T1 | ggcccgtacttcaccg | |
| *orf1* T2 | gtccggggtctgacag | |
| *cypH* T1 | atccgtgaagaattcgaagacga | |
| *cypH* T2 | gcagcacggtcgc | |
| *cypL* T1 | aagttcgaggtggtgcg | |
| *cypL* T2 | cccgcagccagtgg | |
| *cypD* T1 | gatgaagccgatcctcacc | |
| *cypD* T2 | acacccaggcgacgag | |
| *cypM* T1 | cgagttcgcgccgc | |
| *cypM* T2 | gttgcttccggtgaggc | |
| *cypT* T1 | ggtctggaggtgacgg | |
| *cypT* T2 | gtgtacgcccagtcctg | |
| *cypP* T1 | cctggtcgccaccc | |
| *cypP* T2 | catggtgtccatcctgcc | |
| *cypI* T1 | acgacggtgtcctggag | |
| *cypI* T2 | ctcagcagcgggaactc | |
| Primers for complementation constructs | | |
| *cypA* 10257 F | aaaaacatatgactcttacgagcacgaattccgc | NdeI |
| *cypA* 10257 R | aaaaaaagctttcagcagaccaggcagatcg | HindIII |
| *cypH* 10257 F | aaaaacatatgctcgcgggagtgacc | NdeI |
| *cypH* 10257 R | aaaaaaagctttcagtaagcatccatgaaccttc | HindIII |
| *cypL* 10257 F | aaaaacatatgcttactgaccggatcaaggg | NdeI |
| *cypL* 10257 R | aaaaaaagctttcacgcggcggctcccg | HindIII |
| *cypD* 10257 F | aaaaacatatgaacgtggagaagttcgaggg | NdeI |
| *cypD* 10257 R | aaaaaaagctttcactcaccggaacgccc | HindIII |

**Table S1. Cont.**

| Strain | Description | Ref. |
|---|---|---|
| *cypM* 10257 F | aaaaacatatgagtgacccgagcgtgta | NdeI |
| *cypM* 10257 R | aaaaaaagctttcactgccgctccagaacga | HindIII |
| 10257 seq F | acgtccatgcgagtgtcc | |
| 10257 seq R | ccaaacggcattgagcgtc | |
| Primers for fusion protein constructs | | |
| Gat *cypA* F | ggggacaagtttgtacaaaaaagcaggcttagaaaacctgtattttcagggcatgactcttacgagcacgaattccgc | |
| Gat *cypA* R | ggggaccactttgtacaagaaagctgggtttcagcagaccaggcagatcg | |
| Gat *cypD* F | ggggacaagtttgtacaaaaaagcaggcttagaaaacctgtattttcagggcgtgaacgtggagaagttcgaggg | |
| Gat *cypD* R | ggggaccactttgtacaagaaagctgggtttcactcaccggaacgccc | |
| Gat *cypM* F | ggggacaagtttgtacaaaaaagcaggcttagaaaacctgtattttcagggcgtgagtgacccgagcgtgta | |
| Gat *cypM* R | ggggaccactttgtacaagaaagctgggtttcactgccgctccagaacga | |

*Unless otherwise stated, all plasmids generated in this work were maintained in DH5α.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