Electronic Supplementary Information

Watasemycin biosynthesis in *Streptomyces venezuelae*: thiazoline C-methylation by a type B radical-SAM methylase homologue

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Contents

1. Sequence analysis of the *sven0503-sven0517* gene cluster

Table S1. Proposed functions of proteins encoded by genes in the watasemycin biosynthetic gene cluster.

Gene (accession number)	Size (bp)	Homologue (accession number); origin	Identity/ similarity (%)	Proposed function
sven0503 (CCA53790)	1250	SCAB1361 (CBG67360); *S. scabies* 87.22	44/51	AMP-ligase
sven0504 (CCA53791)	1247	Na^+/H^+ antiporter (WP_032767693); *Streptomyces* sp. CNS654	88/91	Na^+/H^+ antiporter
sven0505 (CCA53792)	1947	SCAB1371 (CBG67361); *S. scabies* 87.22	59/80	AfsR-family regulator
sven0506 (CCA53793)	1365	SCAB1381 (CBG67362); *S. scabies* 87.22	61/82	Salicylate synthase
sven0507 (CCA53794)	690	SCAB1401 (CBG67364); *S. scabies* 87.22	57/74	TetR-family regulator
sven0508 (CCA53795)	1212	*Trans*-acting enoyl reductase (AEV87025); *Actinoplanes* sp. SE50/110	36/46	Reductase
sven0509 (CCA53796)	687	SCAB1401 (CBG67364); *S. scabies* 87.22	70/88	TetR-family regulator
sven0510 (CCA53797)	1602	SCAB1411 (CBG67365); *S. scabies* 87.22	71/79	Salicyl-AMP-ligase
sven0511 (CCA53798)	801	SCAB1421 (CBG67366); *S. scabies* 87.22	55/63	Thioesterase
sven0512 (CCA53799)	4545	SCAB1481 (CBG67372); *S. scabies* 87.22	59/68	NRPS
sven0513 (CCA53800)	1710	SCAB1431 (CBG67367); *S. scabies* 87.22	69/82	ABC transporter permease/ATPase
sven0514 (CCA53801)	1842	SCAB1441 (CBG67368); *S. scabies* 87.22	68/79	ABC transporter permease/ATPase
sven0515 (CCA53802)	1605	Radical SAM domain protein (AEV87031); *Actinoplanes* sp. SE50/110	65/77	Radical SAM methyltransferase
sven0516 (CCA53803)	1227	*Trans*-acting enoyl reductase (AEV87025); *Actinoplanes* sp. SE50/110	48/56	Reductase
sven0517 (CCA53804)	5481	SCAB1471 (CBG67371); *S. scabies* 87.22	47/59	NRPS

2. Experimental procedures and additional data

2.1 Strains and plasmids used in this study

Table S2. Strains used in this study.

Strain	Characteristic	Reference
Escherichia coli		
BT340	DH5α/pCP20	1
BW25113	K-12 derivative: Δ*araBAD,* Δ*rhaBAD*	2
ET12567	*dam, dcm, hsdM, hsdS, hsdR, cat, tet*	3
Streptomyces venezuelae		
ATCC 10712	Wild type strain	4
Streptomyces coelicolor		
M1152	M145 Δ*act* Δ*red* Δ*cpk* Δ*cda rpoB[C1298T]*	5
M1152/SV-2_E03::*Ssp*I	SV-2_E03::*Ssp*I in øC31 *attB*	This study
M1152/SV-2_E03::*Ssp*I/Δ*sven0508*	SV-2_E03::*Ssp*I/Δ*sven0508* in øC31 *attB* (Δ*sven0508*)	This study
M1152/SV-2_E03::*Ssp*I/Δ*sven0515*	SV-2_E03::*Ssp*I/Δ*sven0515* in øC31 *attB (*Δ*sven0515)*	This study
M1152/SV-2_E03::*Ssp*I/Δ*sven0516*	SV-2_E03::*Ssp*I/Δ*sven0516* in øC31 *attB (*Δ*sven0516)*	This study

Table S3. Plasmids used in this study.

Plasmid	Characteristic	Reference
SuperCosI	*neo, bla*	Stratagene
pIJ10702	*bla, aac(3)IV, oriT,* øC31 *int-attP*	6
pIJ773	pBS SK+ containing cassette P1-FRT-*oriT-aac(3)IV*-FRT-P2	7
pIJ790	λ-RED (*gam, bet, exo*), *cat, araC, rep101*^{ts}	7
pUZ8002	*tra, neo,* PR4	8
SV-2_E03	SuperCosI containing thiazostatin biosynthetic gene cluster	This study
SV-2_E03/Δ*sven0508*	SV-2_E03 in-frame deletion of *sven0508*	This study
SV-2_E03/Δ*sven0515*	SV-2_E03 in-frame deletion of *sven0515*	This study
SV-2_E03/Δ*sven0516*	SV-2_E03 in-frame deletion of *sven0516*	This study
SV-2_E03::*Ssp*I	SV-2_E03 with pIJ10702 backbone	This study
SV-2_E03::*Ssp*I/Δ*sven0508*	SV-2_E03/Δ*sven0508* with pIJ10702 backbone	This study
SV-2_E03::*Ssp*I/Δ*sven0515*	SV-2_E03/Δ*sven0515* with pIJ10702 backbone	This study
SV-2_E03::*Ssp*I/Δ*sven0516*	SV-2_E03/Δ*sven0516* with pIJ10702 backbone	This study

2.2 PCR targeting

PCR targeting was performed as reported by Gust *et al*.[7] PCR-targeting primer pairs were used to amplify an apramycin resistance cassette containing flippase recombination enzyme (FLP) recognition sites from pIJ773 (Table S3). The resulting cassettes were introduced into *Escherichia coli* BW25113/pIJ790/SV-2_E03 to delete *sven0508*, *sven0515* and *sven0516* in SV-2_E03 by double homologous recombination. The cosmids were introduced into *E. coli* BT340 and the resulting strains incubated at 42 °C to induce expression of the FLP recombinase. The FLP-mediated excision of the apramycin resistance cassette yielded cosmids SV-2_E03/Δ*sven0508*, SV-2_E03/Δ*sven0515* and SV-2_E03/Δ*sven0516*, containing in-frame 'scar' sequences. The integrity of the cosmids was confirmed by PCR analysis using the appropriate primer pairs listed in Table S4 (Figure S1). Derivatives capable of integrating into the chromosome of *Streptomyces* hosts (SV-2_E03::*Ssp*I/Δ*sven0508*, SV-2_E03::*Ssp*I/Δ*sven0515* and SV-2_E03::*Ssp*I/Δ*sven0516*) were made by targeting the cosmids with a 5.2-kb *Ssp*I fragment from pIJ10702, carrying an apramycin resistance gene, *oriT*, a øC31 *attP* site and an integrase gene (*int*).

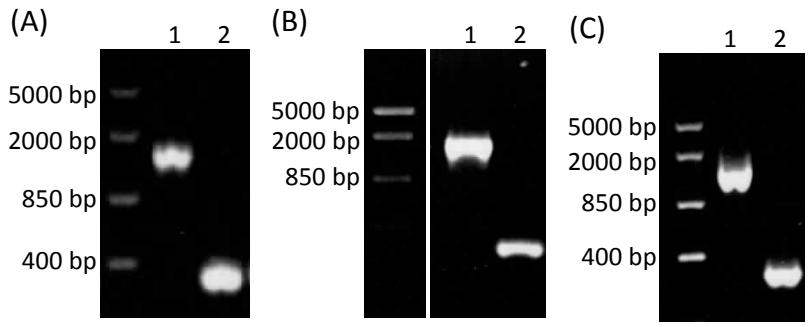


Figure S1. Agarose gel electrophoresis analysis of PCR reactions used to confirm gene deletions. (A) *sven0508* CF and CR primers, 1: SV-2_E03, 2: SV-2_E03/Δ*sven0508*; (B) *sven0515* CF and CR primers, 1: SV-2_E03, 2: SV-2_E03/Δ*sven0515*; (C) *sven0516* CF and CR primers, 1: SV-2_E03, 2: SV-2_E03/Δ*sven0516*.

Table S4. Primers used in this study.

Primer	Sequence
PCR-targeting	
sven0508 F	CTCGGCGGGCGGCGGCCCCACCCGGGAGAACACGCATGAATTCCGGGGATCCGTCGACC
sven0508 R	TCCGCCGCCGCCCGGCCCCGGCACCGGCCGTCCCGCTCATGTAGGCTGGAGCTGCTTC
sven0515 F	GGCGGTCACGACCTCACCACAGGAGAGCTGGAACGCGTGATTCCGGGGATCCGTCGACC
sven0515 R	CGGCCCGCCGCGCCGCCCTGCTCCTCCGCTCCCGGTTCATGTAGGCTGGAGCTGCTTC
sven0516 F	GCCGTGGCCCGCGCACTGCTCGCGGAGGACGGCCGGTGAATTCCGGGGATCCGTCGACC
sven0516 R	AGCCCGCCGCGCCGTCGCGCCGCCGACCGGAAGACGTCATGTAGGCTGGAGCTGCTTC
Confirmation of gene-deletion	
sven0508 CF	CACCCAGCGCGAGCAGGTCTTC
sven0508 CR	GGTGCGTCGGGTCTGAGGTGTC
sven0515 CF	GATCTGAGGCGCGGAGAATC
sven0515 CR	TGGAGGAAGGGTGTGCTCTG
sven0516 CF	TGTGCGACCCGGTCTGAAC
sven0516 CR	CCGCGACATGACCGACTACTG

2.3 Intergeneric conjugation from E. coli *to* S. coelicolor *M1152*

Integrative cosmids were introduced into *E. coli* ET12567/pUZ8002 by electroporation. An ampicillin-resistant colony containing each of the cosmids was used to transfer the plasmid from *E. coli* to *S. coelicolor* M1152 by intergeneric conjugation on SFM agar (soyflour 2.0%, mannitol 2.0%, agar 2.0%, tap water) following the method of Kieser *et al.*[9] This procedure yielded the following apramycin resistant exconjugants: *S. coelicolor* M1152/SV-2_E03::*Ssp*I, *S. coelicolor* M1152/ SV-2_E03::*Ssp*I/Δ*sven0508*, *S. coelicolor* M1152/SV-2_E03::*Ssp*I/Δ*sven0515* and *S. coelicolor* M1152/ SV-2_E03::*Ssp*I/Δ*sven0516.*

2.4 Isolation and structural characterisation of thiazostatin and watasemycin

S. coelicolor M1152/SV-2_E03::*Ssp*I was cultured in 2 L of YD medium (yeast extract 1.0%, glucose 1.0%) shaken at 180 rpm and 30 °C for 5 days. The culture broth was separated from the mycelium by centrifugation at 5,000 rpm and added to 30 mL of Diaion HP-20 (Sigma), pre-swollen with ethanol. The resulting mixture was shaken at

180 rpm for 2 hours. The resin was packed in a column and washed with 100 mL of 50% methanol, and then eluted with 200 mL of methanol. The eluate was concentrated *in vacuo*, and the residual aqueous solution was extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness *in vacuo* and the residue (45 mg) was purified by HPLC on an Agilent C18 column (21 x 100 mm) connected to an Agilent 1100 instrument, eluting with 50% acetonitrile/0.1% formic acid at 5 mL/min and monitoring absorbance at 210 nm. The fractions eluting at 16.8, 20.1, 24.5 and 27.0 minutes were adjusted to pH 7.0 with 1 M Na_2CO_3 and concentrated *in vacuo* to remove acetonitrile. The resulting solutions were adjusted to pH 5.0 with HCl and extracted with ethyl acetate. Each organic extract was concentrated to dryness *in vacuo* to yield wataemycin A (5 mg), thiazostatin B (1 mg), watasemycin B (5 mg) and thiazostatin A (1 mg). The ^{1}H NMR spectra (400 MHz) of these compounds are shown in Figures S2-S5.

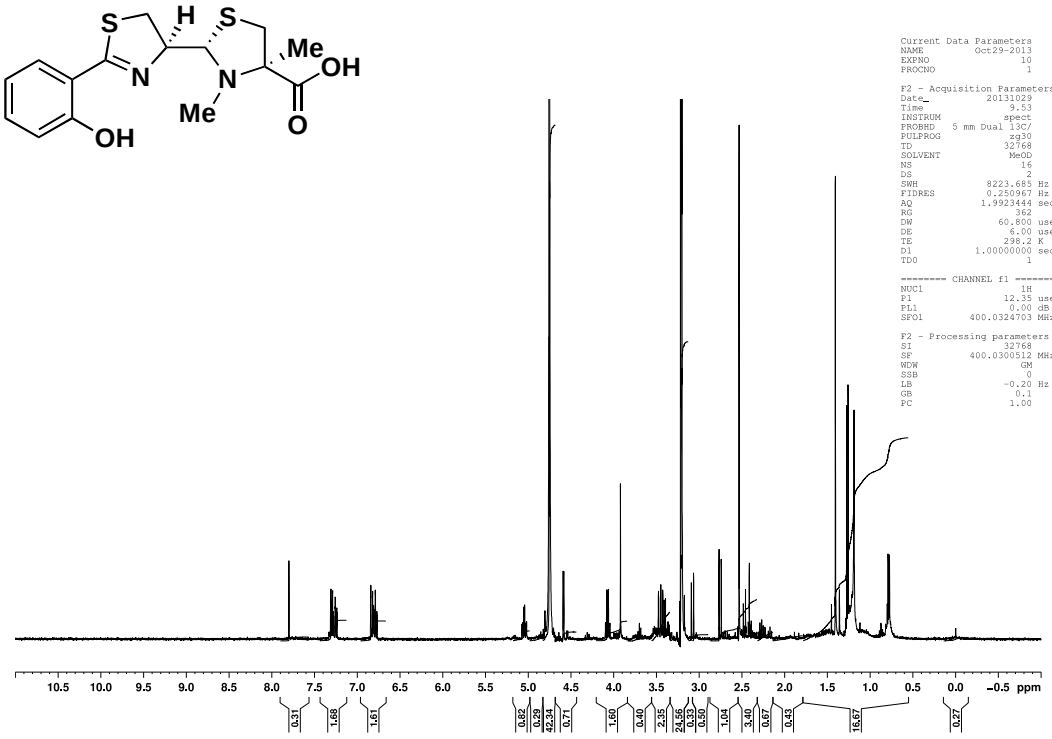


Figure S2. ^{1}H NMR spectrum of thiazostatin A (in CD_3OD).

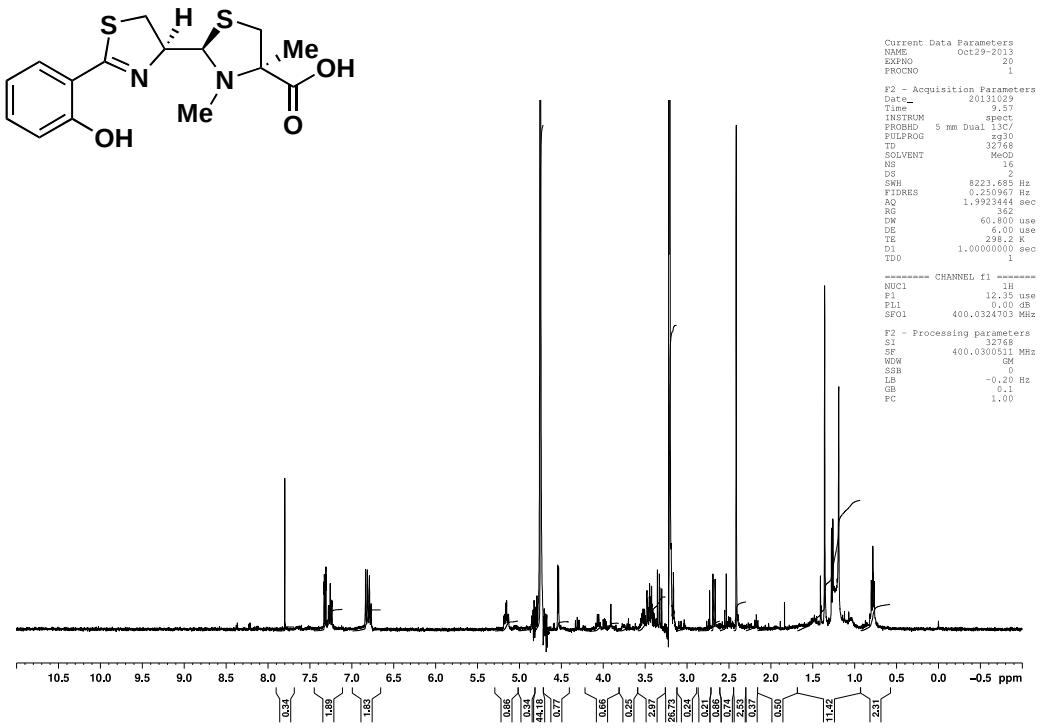


Figure S3. ^{1}H NMR spectrum of thiazostatin B (in CD_3OD).

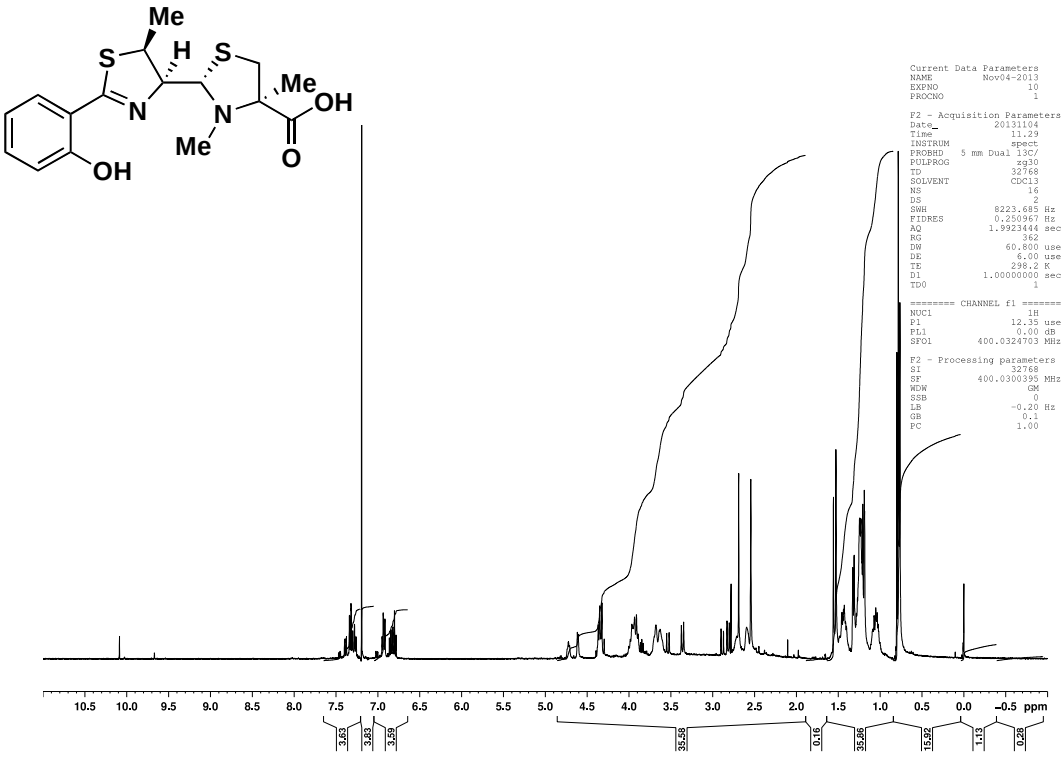


Figure S4. ^{1}H NMR spectrum of watasemycin A (in $CDCl_3$).

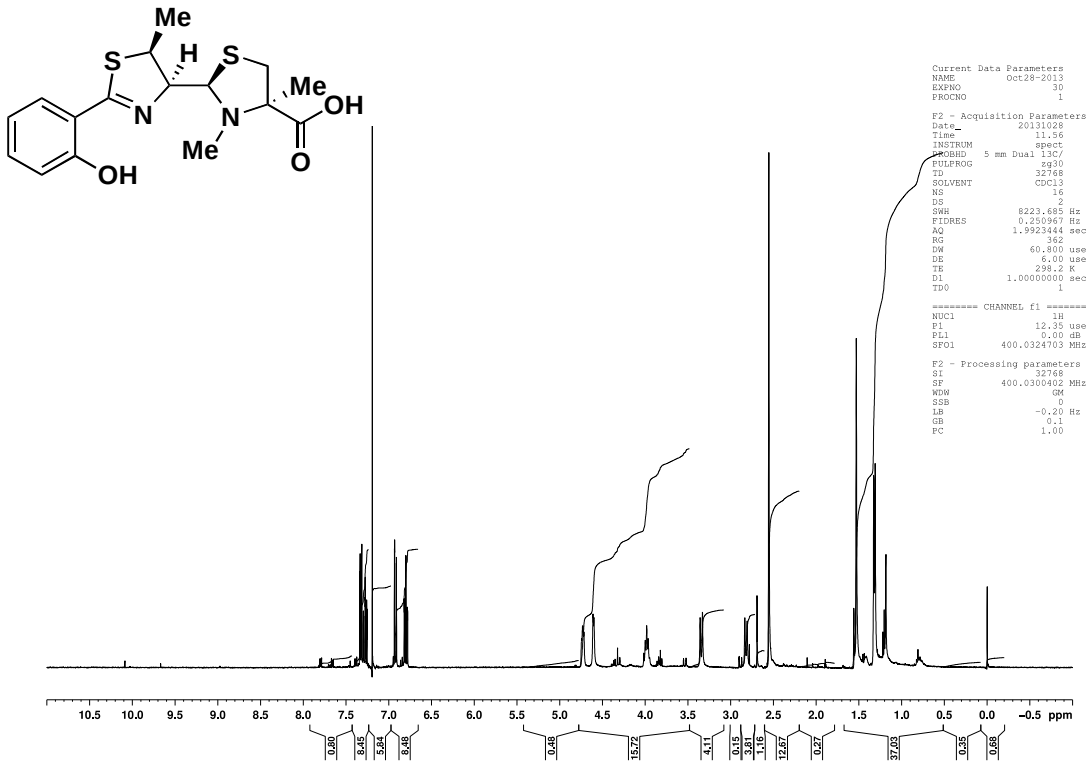


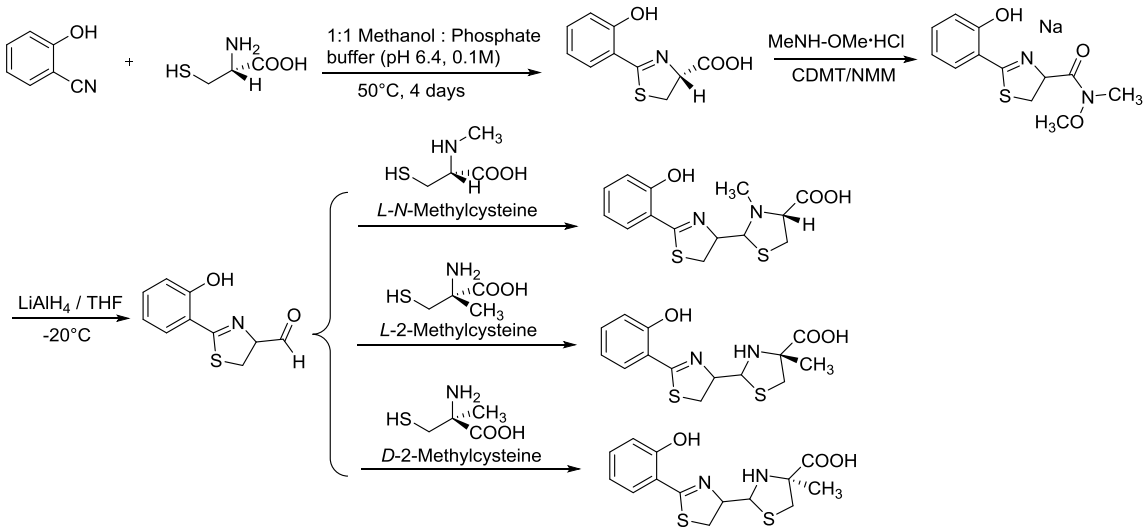
Figure S5. ^{1}H NMR spectrum of watasemycin B (in $CDCl_3$).

2.5 Synthesis of pyochelin and isopyochelin

General information

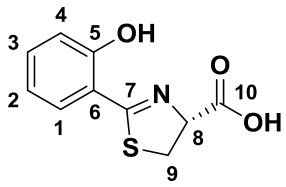
All chemicals were purchased from commercial sources and were used as received. ^{1}H and ^{13}C NMR spectra were measured at 300 MHz and 75 MHz, respectively. All chemical shifts are given as δ values in ppm with reference to residual chloroform (δ_H 7.26), the central peak of chloroform-d (δ_C 77.00) or DMSO-d_6 (δ_C 39.52), as appropriate. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, t = triplet, dd = doublet of doublets, td = triplet of doublets, m = multiplet, br = broad), integration, assignment and coupling constant (Hz). HRMS spectra were measured on a Bruker MaXis impact mass spectrometer. Melting points were measured using a Stuart SMP10 melting point apparatus and are uncorrected.

The synthetic route employed is outlined in Scheme S1.[10]



Scheme S1. Route employed for the synthesis of pyochelin and isopyochelin.

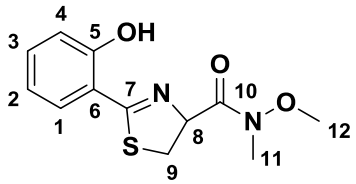
(R)-2-(2-Hydroxyphenyl)-4,5-dihydrothiazole-4-carboxylic acid



A solution of 2-hydroxy-2-benzonitrile (1.90 g, 16.0 mmol) and L-cysteine (3.86 g, 31.9 mmol) in a 1:1 (v/v) mixture of methanol and phosphate buffer pH 6.4 (75 mL) was stirred under an argon atmosphere at 50 °C for 4 days. A precipitate formed that was removed by filtration and the filtrate was concentrated under vacuum. 20 mL of water and 10 mL of 1 M HCl solution was added to the aqueous residue and the mixture was extracted with dichloromethane. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated under vacuum. The residue was recrystallized using a mixture of ethanol and hexane to yield the product (2.85 g, 80%) as a yellow solid, mp 114 - 116 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.61 - 3.73 (m, 2H, H-9), 5.42 (dd, 1H, H-8, J = 9.3 and 8.1 Hz), 6.86 - 6.92 (m, 1H, H-4), 7.03 (dd, 1H, H-2, J = 8.3 and 0.6 Hz), 7.35 - 7.39 (m, 1H, H-3), 7.43 (dd, 1H, H-1, J = 8.0 and 1.4 Hz), 10.79 (br s, 2H, O-H and COO-H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 172.71 (C-10), 171.29 (C-7), 158.38 (C-5), 133.72 (C-3), 130.53 (C-1), 119.34 (C-2), 116.87 (C-4),

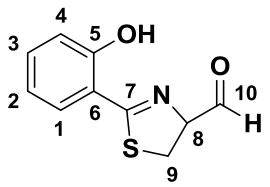
115.60 (C-6), 76.32 (C-8), 33.42 (C-9); HR-MS: *m/z* calculated for $C_{10}H_{10}NO_3S$ $[M+H]^+$: 224.0381, found: 224.0378.

2-(2-Hydroxyphenyl)-N-methoxy-N-methyl-4,5-dihydrothiazole-4-carboxamide



To a solution of (*R*)-2-(2-hydroxyphenyl)-4,5-dihydrothiazole-4-carboxylic acid (2.85 g, 12.8 mmol) in 30 mL THF was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (2.70 g, 15.4 mmol) and *N*-methylmorpholine (4.2 mL, 38.2 mmol) at 0 °C. The resulting mixture was stirred for 1 hour and *N,O*-dimethylhydroxylamine hydrochloride (1.25 g, 12.8 mmol) was added. After stirring at room temperature overnight, 50 mL of water was added. The THF was removed under vacuum, and the remaining aqueous solution was acidified to pH 4 and extracted with dichloromethane. The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (pet ether/EtOAc 2:1) to yield the product (1.43 g, 42%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (s, 3H, H-11), 3.46 (dd, 1H, H-9a, *J* = 10.9 and 9.3 Hz), 3.75 (t, 1H, H-9b, *J* = 10.0 Hz), 3.81 (s, 3H, H-12), 5.68 (t, 1H, H-8, *J*= 9.0 Hz), 6.86 (td, 1H, H-4, *J* = 7.5 and 1.1 Hz), 6.97 (dd, 1H, H-2, *J* = 8.3 and 0.9 Hz), 7.31 - 7.37 (m, 1H, H-3), 7.41 (dd, 1H, H-1, *J* =7.8 and 1.5 Hz), 12.32 (br s, 1H, O-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.98 (C-10), 169.62 (C-7), 158.87 (C-5), 133.25 (C-3), 130.71 (C-1), 118.86 (C-2), 116.96 (C-4), 116.03 (C-6), 74.51 (C-8), 61.72 (C-12), 32.76 (C-9), 32.45 (C-11); HR-MS: *m/z* calculated for $C_{12}H_{14}N_2NaO_3S$ $[M+Na]^+$: 289.0617, found: 289.0618.

2-(2-Hydroxyphenyl)-4,5-dihydrothiazole-4-carbaldehyde



To a solution of 2-(2-hydroxyphenyl)-N-methoxy-N-methyl-4,5-dihydrothiazole-4-carboxamide (0.45 g, 1.7 mmol) in 20 mL dry Et_2O at -20 °C was added $LiAlH_4$ (0.19 g, 5.0 mmol). The resulting mixture was stirred at -20 °C until the starting material had been consumed, and 2 mL methanol, 20 mL saturated NH_4Cl solution and 10 mL 5% (v/v) H_2SO_4 solution were successively added. The organic phase was collected and the aqueous phase was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated under vacuum to yield the crude aldehyde (0.21 g, 60%) as a yellow oil. The aldehyde was unstable and therefore immediately used in the next step without further purification. HR-MS: *m/z* calculated for $C_{10}H_{10}NO_2S$ $[M+H]^+$: 208.0427, found: 208.0432.

Pyochelin/neopyochelin

To a solution of 2-(2-hydroxyphenyl)-4,5-dihydrothiazole-4-carbaldehyde (25 mg, 0.12 mmol) in a mixture of 5 mL ethanol and 1.3 mL water was added CH_3COOK (82 mg, 0.84 mmol) and the hydrochloride salt of L-N-methylcysteine (30 mg, 0.17 mmol). After stirring at room temperature for 24 hours, 15 mL of water was added and the mixture washed with hexane. The aqueous phase was acidified to pH 5 and extracted with EtOAc. The ethyl acetate extract was washed with brine, dried over $MgSO_4$ and concentrated under vacuum to yield pyochelin/neopyochelin as a yellow solid consisting of four inseparable diastereomers that were subjected directly to comparative LC-MS analyses.

Isopyochelin

The same procedure as that employed for the synthesis of pyochelin/neopyochelin was utilized, except that L- and D-2-methylcysteine were used in place of L-N-methylcysteine. In both cases and inseparable mixture of four diastereomeric products was obtained that was subjected directly to LC-MS analyses.

2.6 LC-MS analyses

Sample preparation

Strains of *S. coelicolor* M1152/SV-2_E03*::Ssp*I and the Δ*sven0508*, Δ*sven0515* and Δ*sven0516* mutants were grown in liquid YD medium. Seed cultures were prepared in 50 mL of YD medium inoculated with 20 μL of spores. After incubation for 2 days at 180 rpm and 30 °C, 2% of this culture was used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of YD medium. The resulting cultures were grown for 5 days at 180 rpm and 30 ºC. An equal volume of ethanol was added to each culture and the resulting mixtures were shaken for 1 hour at 180 rpm. The insoluble materials were pelleted at 13200 rpm for 10 min and the supernatant was passed through a 0.4 μm filter immediately prior to LC-MS analysis.

For the experiment in which the extract of the Δ*sven0515* mutant was fed to the Δ*sven0516* mutant, a 5-day-old culture (50 mL) of the Δ*sven0515* mutant was first centrifuged at 5,000 rpm for 10 min to remove the mycelium. Then the supernatant was acidified with 1M HCl to pH 5.0 and extracted with EtOAc. The EtOAc extract was dried over $MgSO_4$ and concentrated under vacuum. The residue containing the metabolites from the culture of the Δ*sven0515* mutant was dissolved in 3 mL of YD medium and fed to a 50 mL culture of the Δ*sven0516* mutant, which had been incubated for 1 day after inoculation with 2% of the seed culture. The resulting culture was grown for 4 days at 180 rpm and 30 °C, and extracts for LC-MS analyses were prepared as described above.

Instrumentation and elution conditions

LC-MS analyses were performed on a Dionex Ultimate 3000 RS UHPLC instrument equipped with ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm) coupled to a Bruker MaXis Impact mass spectrometer [ESI in positive ion mode; full scan 50-2500 *m/z*; end plate offset, -500 V; capillary, -4500 V; nebulizer gas (N_2), 1.4 bar; dry gas (N_2), 8 L/min; dry temperature, 200 °C]. The solvents used for elution of the column were as follows: solvent A, 0.1% formic acid in water; solvent B, 0.1% formic acid in acetonitrile. The elution profile was as follows: 0-5 min, 5% B; 5-17 min, linear gradient from 5% B to 100% B; 17-22 min, 100% B; 22-25 min, linear gradient from 100% B to 5% B; 25-34 min, 5% B. The flow rate was 0.2 mL/min. The mass spectrometer was calibrated with 10 mM sodium formate at the beginning of each run.

An Agilent 1260 Infinity HPLC coupled to a Bruker AmaZon X mass spectrometer [ESI in positive ion mode; full scan 100-3000 *m/z*; end plate offset, -500 V; capillary, -4500 V; nebulizer gas (N_2), 10 p.s.i.; dry gas (N_2), 4 L/min; dry temperature, 180 °C] was used for the analysis of isopyochelin samples on a homochiral stationary phase. Synthetic isopyochelin and the ethanol extract of the *S. coelicolor* M1152/SV-2_E03::*Ssp*I culture broth were analyzed on a ChiralPAK IE analytical column (4.6 × 150 mm, 5 μm) in reverse phase mode. The solvents used for elution of the column were as follows: solvent A, 0.1% formic acid in water; solvent B, 0.1% formic acid in acetonitrile. The elution profile was as follows: 0-5 min, 20% B; 5-30 min, linear gradient from 20% B to 100% B; 30-35 min, 100% B; 35-40 min, linear gradient from 100% B to 20% B. The flow rate was 0.8 mL/min.

Results of analyses

Metabolites with molecular formulae corresponding to pyochelin in the ethanol extract of the *S. coelicolor* M1152/SV-2_E03::*Ssp*I culture broth had different retention times from the four synthetic stereoisomers of pyochelin (Figure S6).

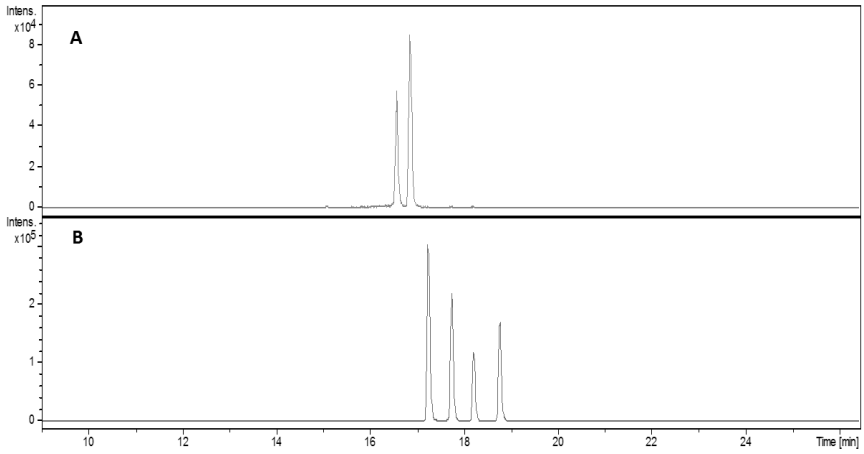


Figure S6. Extracted ion chromatograms at *m/z* = 325.0675, corresponding to $[M+H]^+$ for pyochelin/isopyochelin from the ethanol extract of the *S. coelicolor* M1152/SV-2_E03::*Ssp*I culture broth (A) and the synthetic mixture of pyochelin and neopyochelin (B). Peak doubling results from epimerization at C-2", which gives rise to two diastereomers for each compound.

LC-MS analyses on a homochiral stationary phase established the absolute configuration at C-4" of isopyochelin (Figure S7). Two of the synthetic stereoisomers of isopyochein derived from D-2-methylcysteine had the same retention times as the natural product. This indicates that the stereochemistry at C-4" of isopyochelin corresponds to D-2-methylcysteine and thus is *S*.

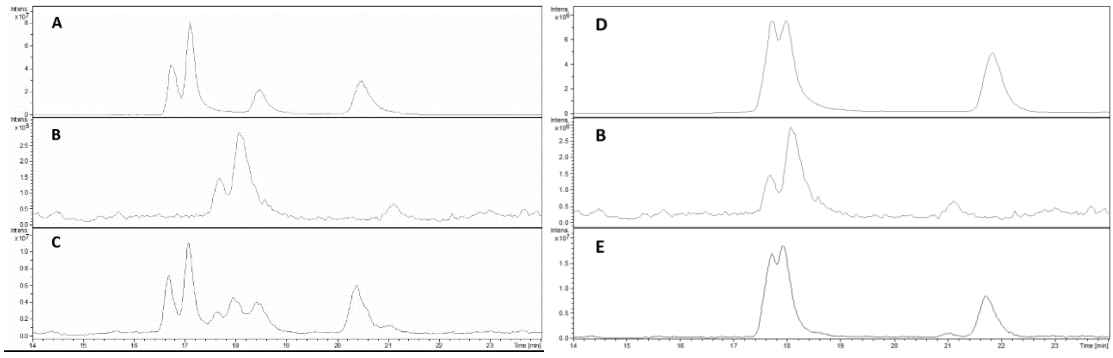


Figure S7. Extracted ion chromatograms at *m/z* = 325.0 (corresponding to $[M+H]^+$ for isopyochelin) from LC-MS analyses on a homochiral stationary phase of: (A) Synthetic stereoisomers of isopyochelin derived from L-2-methylcysteine; (B) the ethanol extract

of the *S. coelicolor* M1152/SV-2_E03::*Ssp*I culture broth; (C) a mixture of the above two samples; (D) Synthetic stereoisomers of isopyochelin derived from D-2-methylcysteine; (E) a mixture of the samples in B and D. Natural isopyochelin exists as a mixture of two diastereomers as a result of rapid epimerization at C-2".

Analysis of ethanol extracts of the Δ*sven0508 and* Δ*sven0516 mutant strains*

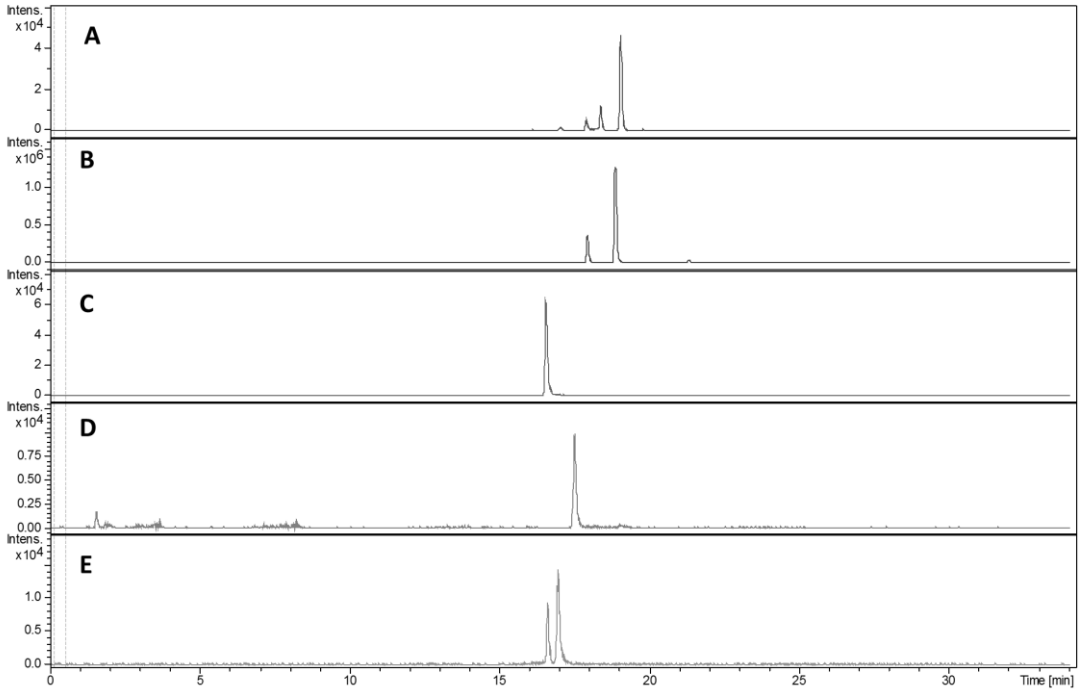


Figure S8. Extracted ion chromatograms at *m/z* = 339.0832 (A), 353.0988 (B), 210.0583 (C), 224.0740 (D) and 325.0675 (E), corresponding to $[M+H]^+$ for thiazostatin, watasemycin, aerugine, pulicatin and isopyochelin, respectively, from LC-MS analyses of the ethanol extract of the Δ*sven0508* mutant culture broth. Peak doubling results from epimerization at C-2", which gives rise to two diastereomers for thiazostatin, watasemycin and isopyochelin. The chromatograms show that production of all of the 2-hydroxyphenylthiazoline-containing metabolites was unaffected by deletion of *sven0508*.

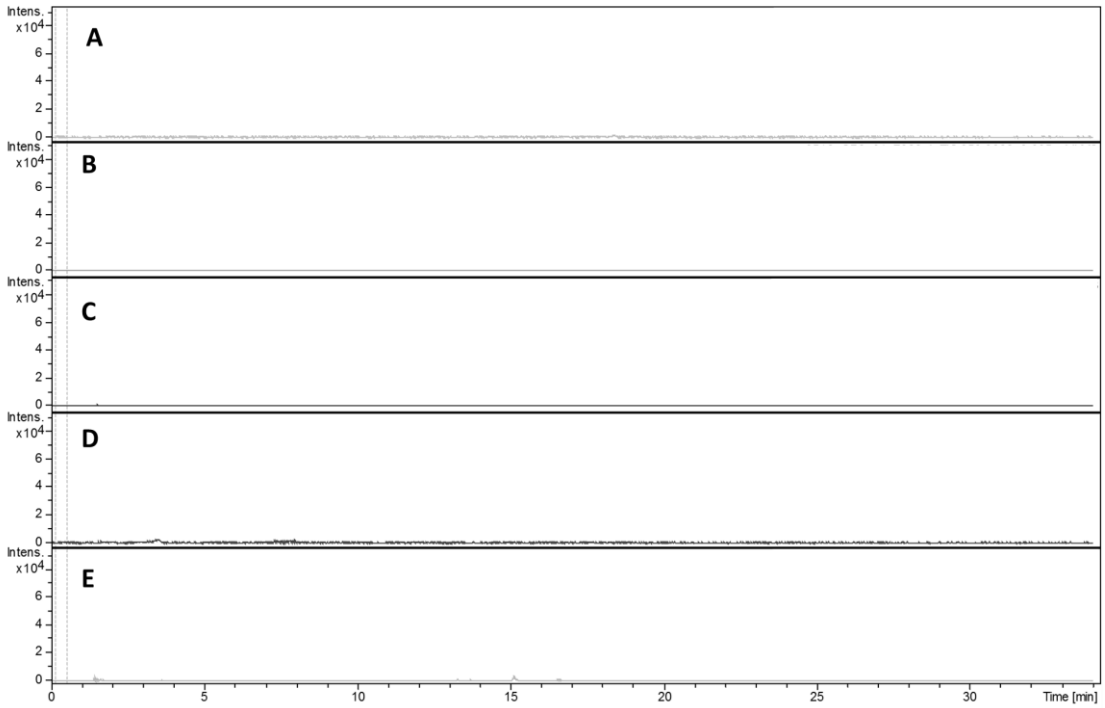


Figure S9. Extracted ion chromatograms at *m/z* = 339.0832 (A), 353.0988 (B), 210.0583 (C), 224.0740 (D) and 325.0675 (E), corresponding to $[M+H]^+$ for thiazostatin, watasemycin, aerugine, pulicatin and isopyochelin, respectively, from LC-MS analyses of the ethanol extract of the Δ*sven0516* mutant culture broth. The chromatograms show that production of all of the 2-hydroxyphenylthiazoline-containing metabolites is abolished by the deletion of *sven0516*.

LC-MS/MS analysis of thiazostatin, watasemycin and their putative methyl ester derivatives

An additional peak with a retention time of approximately 21.5 minutes can be observed in the extracted ion chromatograms at *m/z* = 353.0988, corresponding to $[M+H]^+$ for watasemycin, from LC-MS analyses of the ethanol extract of *S. coelicolor* M1152/SV-2_E03::*Ssp*I and the Δ*sven0515* mutant (Figures 5B and 7B in the main manuscript). Comparative LC-MS/MS analyses of thiazostatin, watasemycin and this additional compound indicate that it is the methyl ester of thiazostatin (Figure S10). Similarly, the methyl ester of watasemycin can also be observed in the ethanol extract of *S. coelicolor*

M1152/SV-2_E03::*Ssp*I (Figure S10).

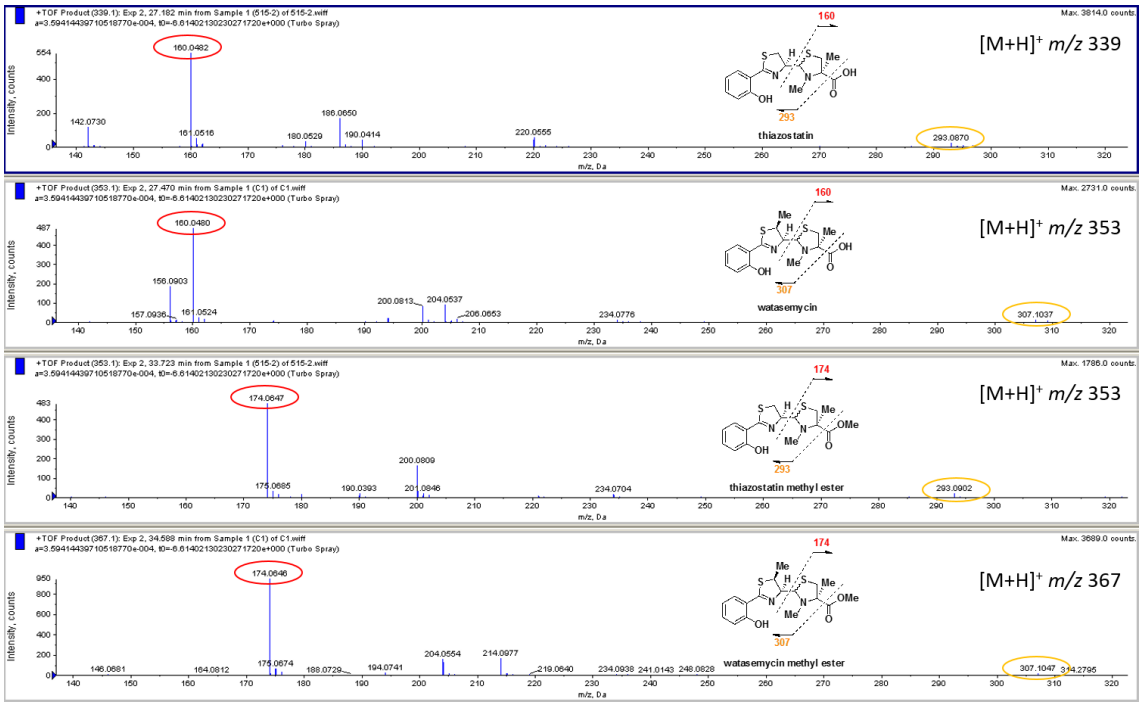


Figure S10. Spectra from LC-MS/MS analyses of thiazostatin, watasemycin and their putative methyl ester derivatives.

2.7 Analysis of the expression profiles of genes flanking the cluster

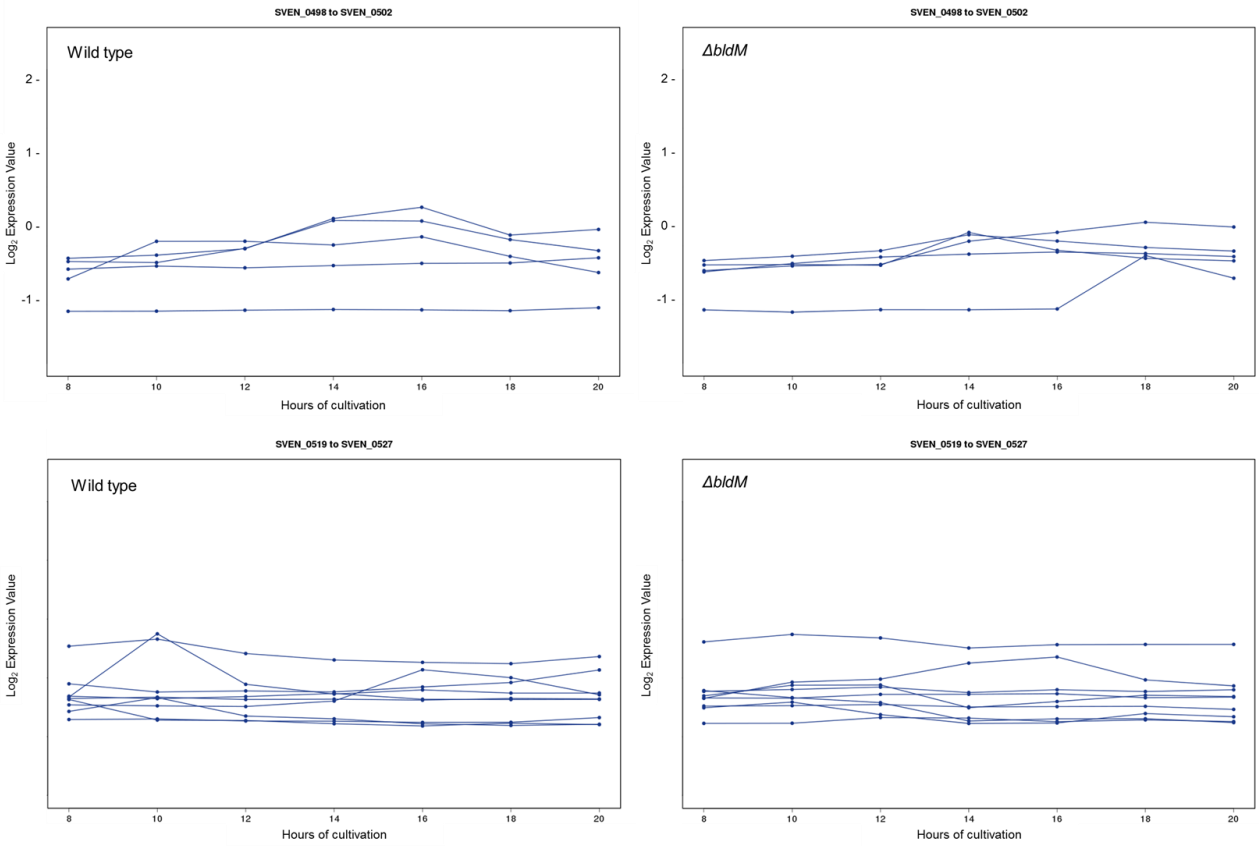


Figure S11. Microarray expression profiles of the genes flanking the watasemycin biosynthetic gene cluster (*sven0498–sven0502,* top; *sven0519–sven0527,* bottom). Basal levels of expression are observed throughout growth in both the *S. venezuelae* wild type strain (left panel) and the *bldM* mutant (right panel). The y-axis represents normalized transcript abundance.

3. References

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