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

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2-Hydroxyphenylthiazolines are a family of iron-chelating nonribosomal peptide natural products that function as virulence-conferring siderophores in various Gram-negative bacteria. They have also been reported as metabolites of Gram-positive *Streptomyces* species. Transcriptional analyses of *Streptomyces venezuelae* ATCC 10712 revealed that its genome contains a putative 2-hydroxyphenylthiazoline biosynthetic gene cluster. Heterologous expression of the gene cluster in *Streptomyces coelicolor* M1152 showed that the mono- and dimethylated derivatives, thiazostatin and watasemycin, respectively, of the 2-hydroxyphenylthiazoline enantiopyochelin are two of its metabolic products. In addition, isopyochelin, a novel isomer of pyochelin containing a C-methylated thiazolidine, was identified as a third metabolic product of the cluster. Metabolites with molecular formulae corresponding to aerugine and pulicatin A/B were also detected. The structure and stereochemistry of isopyochelin were confirmed by comparison with synthetic standards. The role of two genes in the cluster encoding homologues of PchK, which is proposed to catalyse thiazoline reduction in the biosynthesis of enantiopyochelin in *Pseudomonas protegens*, was investigated. One was required for the production of all the metabolic products of the cluster, whereas the other appears not to be involved in the biosynthesis of any of them. Deletion of a gene in the cluster encoding a type B radical-SAM methylase homologue abolished the production of watasemycin, but not thiazostatin or isopyochelin. Feeding of thiazostatin to the mutant lacking the functional PchK homologue resulted in complete conversion to watasemycin, demonstrating that thiazoline C-methylation by the type B radical-SAM methylase homologue is the final step in watasemycin biosynthesis.

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## Introduction

2-Hydroxyphenylthiazolines are a family of bacterial natural products with a variety of biological activities. Some members of this family function as siderophores, iron chelators produced for iron uptake that are virulence factors in numerous pathogens.<sup>1–3</sup> Examples include yersiniabactin **1** produced by enteropathogenic *Yersinia* species,<sup>4</sup> anguibactin **2** from *Vibrio anguillarum* 775, which causes hemorrhagic septicemia in

fish,<sup>5,6</sup> and pyochelin **3** and aerugine **4** produced by *Pseudomonas* species including *Pseudomonas aeruginosa*, an opportunistic human pathogen (Fig. 1).<sup>7,8</sup>

Intriguingly, the non-pathogenic pseudomonad *Pseudomonas protegens* produces enantiopyochelin **5**, which has also been shown to function as a siderophore (Fig. 1).<sup>9</sup> Other members of the 2-hydroxyphenylthiazoline family are produced by non-pathogenic Actinobacteria. These include thiazostatin **6**, watasemycin **7** and the pulicatin **8–12** (Fig. 1), produced by *Streptomyces* species, which have been reported to have anti-oxidant, antibacterial and neuroactive properties, respectively.<sup>10–12</sup> However, it is not currently known whether these compounds are also able to function as siderophores.

Pyochelin **3** biosynthesis in *P. aeruginosa* is well characterized.<sup>13–18</sup> Seven proteins (PchABCDEFG) assemble pyochelin **3** from salicylate and two molecules of L-cysteine (Fig. 2). Salicylate is generated from chorismate by PchA and PchB. It is then activated by reaction with ATP, catalysed by the standalone adenylation (A) domain PchD. The resulting salicyl adenylate reacts with the phosphopantetheine thiol of the N-terminal peptidyl carrier protein (PCP) domain of the PchE

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Fig. 1 Bacterial natural products belonging to the 2-hydroxyphenylthiazoline family.

nonribosomal peptide synthetase (NRPS) to form the corresponding salicyl thioester. The A domain of PchE similarly loads L-cysteine onto the C-terminal PCP domain of PchE and the heterocyclisation (Cy) domain catalyses the condensation of the salicyl thioester with the cysteinyl thioester to form a 2-hydroxyphenylthiazolinyl thioester. Epimerisation of the cysteine-derived stereocentre in this intermediate is catalysed by the methyl transferase-like ( $MT_e$ ) domain of PchE. The A domain of PchF then loads a second molecule of L-cysteine onto its PCP domain and the Cy domain catalyses condensation of the resulting cysteinyl thioester with the PchE-bound 2-hydroxyphenylthiazolinyl thioester to generate a second thiazoline, which is reduced to the corresponding thiazolidine by PchG and N-methylated by the MT domain of PchF. Hydrolytic release of pyochelin 3 from PchF is catalysed by the C-terminal thioesterase (TE) domain. PchC is a type II TE that removes incorrectly loaded molecules from the PCP domain of PchE and PchF.<sup>18</sup>

Enantiopyochelin 5 biosynthesis in *P. protegens* involves homologues of PchABCDEF (Fig. 2).<sup>9</sup> The PchE homologue lacks the  $MT_e$  domain responsible for epimerisation of the stereocentre in the first thiazoline ring and a homologue of *pchG*, which encodes the thiazoline reductase utilized by *P. aeruginosa*, is not present in the enantiopyochelin biosynthetic gene cluster. Instead, the product of *pchK* is proposed to catalyse reduction of the second thiazoline after the configuration of the C-4'' stereocentre has been inverted by a hitherto unidentified enzyme. The absence of the  $MT_e$  domain in the PchE homologue and the substitution of PchG with PchK are together able to account for the production of enantiopyochelin 5 by *P. protegens*.

The plant pathogen *Streptomyces scabies* 87.22 has also been shown to produce pyochelin 3 in an iron-deficient medium.<sup>19</sup> The gene cluster responsible for its biosynthesis encodes homologues of PchCDEFG (a single enzyme encoded by the *scab1381* gene is proposed to be responsible for salicylate biosynthesis in *S. scabies*) (Fig. 2).<sup>19</sup> Transcription of the gene cluster is repressed by a TetR-family protein encoded by *scab1401* and activated by an AfsR-family protein encoded by *scab1371*.<sup>19</sup>

Here we report the identification of a putative 2-hydroxyphenylthiazoline biosynthetic gene cluster in the genome of *Streptomyces venezuelae* ATCC 10712 by comparative transcriptional analyses of the wild type strain and a *blmD* mutant. The main metabolic products of this cluster were identified as the known 2-hydroxyphenyl-thiazolines thiazostatin 6 and watasemycin 7, and a novel metabolite isopyochelin 13 via a heterologous expression approach. Gene deletion experiments defined an essential role for a PchK homologue in the biosynthesis of all metabolic products of the cluster, and showed that a type B radical-SAM methylase homologue is responsible for methylation of the thiazoline ring in thiazostatin 6 to form watasemycin 7.

## Results and discussion

### Microarray analysis of *S. venezuelae blmD* mutant

We recently reported the results of DNA microarray analyses of *S. venezuelae*, which showed that the transcription of the chloramphenicol biosynthetic gene cluster is markedly increased in a *blmD* mutant relative to the wild type strain.<sup>20</sup> The *blmD* gene encodes an atypical orphan response regulator required for morphological differentiation.<sup>21</sup> Analysis of the same microarray



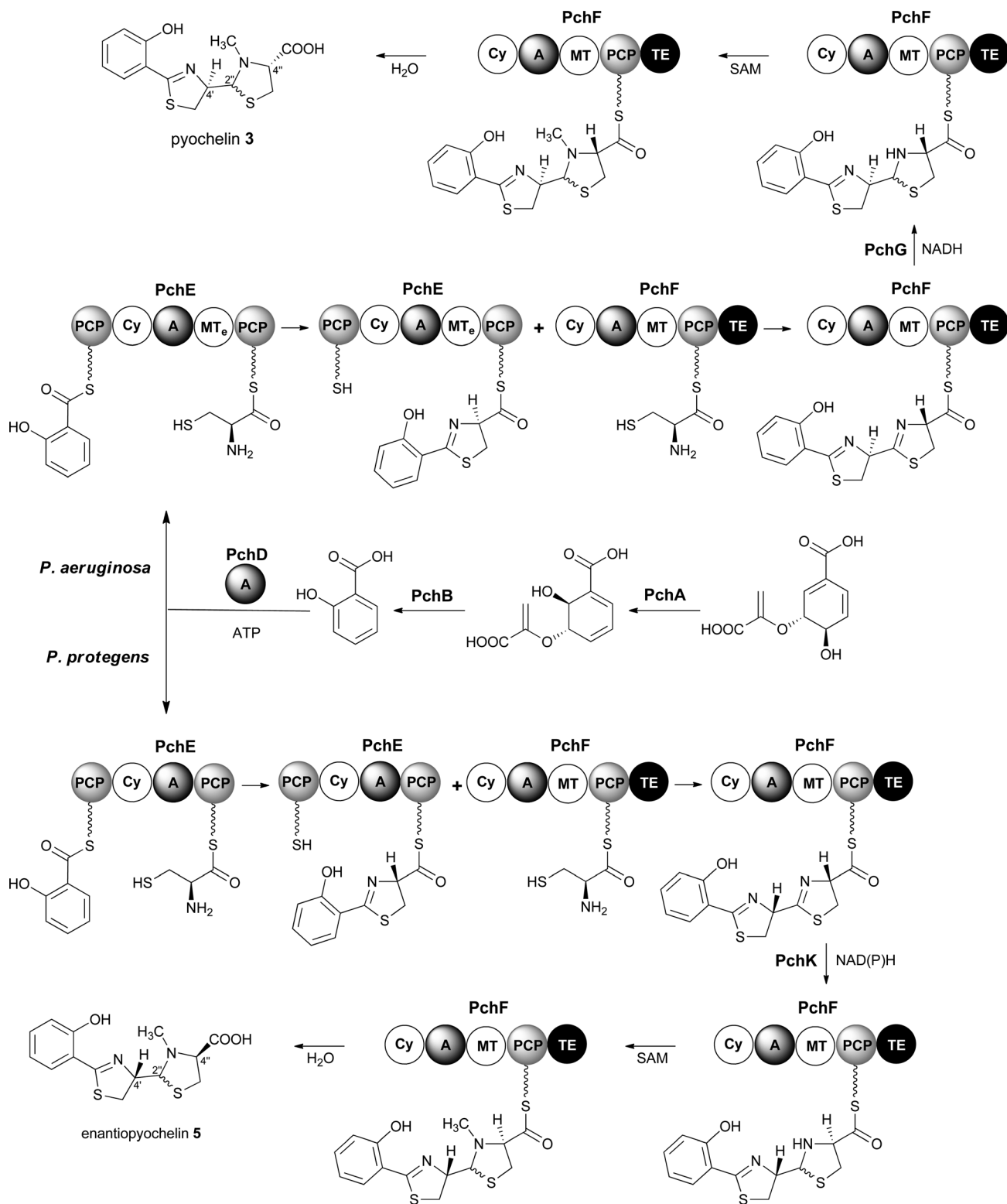
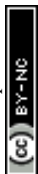


Fig. 2 Pathways proposed for the biosynthesis of pyochelin 3 and enantiopyochelin 5 in *P. aeruginosa* and *P. protegens*, respectively. The MT<sub>e</sub> domain in PchE from *P. aeruginosa* is an MT-like domain that catalyses epimerisation of the  $\alpha$ -carbon in the cysteinyl thioester.

data set revealed another cluster of co-ordinately regulated genes, *sven0503–sven0517*, that is up-regulated in the *blbM* mutant (Fig. 3). The transcriptional profiles of the flanking genes

*sven0498–sven0502* and *sven0519–sven0527* (*sven0518* was not represented on the microarray), which showed basal levels of expression throughout growth in both the wild type strain and



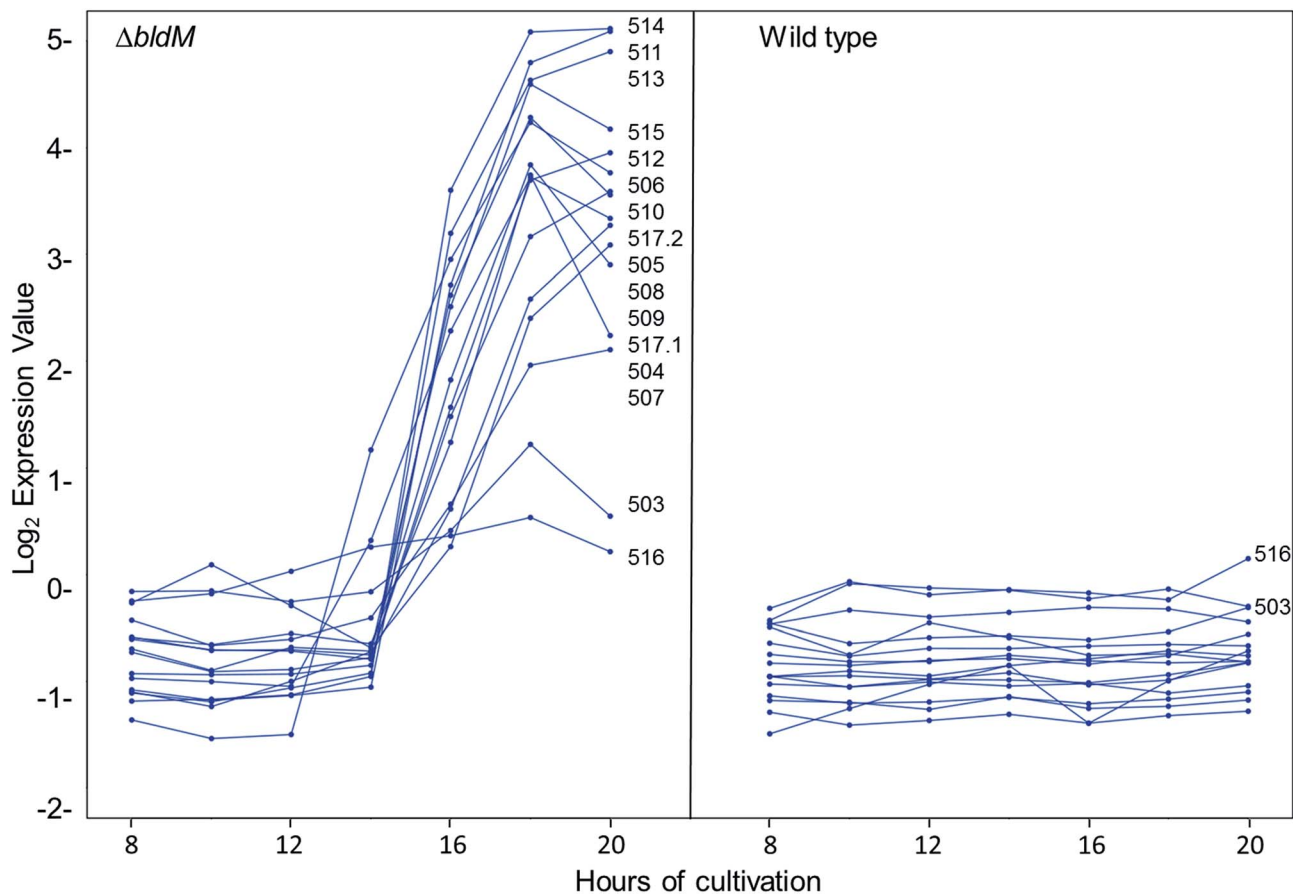


Fig. 3 Microarray expression profiles of the fifteen genes (*sven0503*–*sven0517*) with elevated levels of transcription in the *S. venezuelae* *bldM* mutant (left panel) compared to the wild type strain (right panel). The y-axis represents normalized transcript abundance. The genes are listed in order of their relative levels of expression at 20 hours in the *bldM* mutant (and for *sven0503* and *sven0516* in the wild type strain for comparison). Note that there are two sets of data for *sven0517* (*sven0517.1* and *sven0517.2*) reflecting two different probe sets on the array and that their expression profiles are very similar. The *sven0518* gene, which encodes a conserved 51 amino acid hydrophobic peptide, was not represented on the microarrays and thus its expression profile is not shown.

the *bldM* mutant (see ESI<sup>†</sup>), indicated that they are unlikely to be functionally related to *sven0503*–*sven0517* (note also that a leucyl-tRNA gene, currently lacking a *sven* designation, lies between *sven0517* and *sven0518*, further suggesting that *sven0517* lies at the right hand end of the gene cluster).

#### Sequence analysis of the *sven0503*–*sven0517* gene cluster

Most of the *sven0503*–*sven0517* genes encode proteins with >50% similarity to those encoded by genes within the *S. scabies* pyochelin biosynthetic gene cluster (Fig. 4 and ESI<sup>†</sup>). *Sven0510*, *Sven0512* and *Sven0517* are homologues of *Scab1411* (PchD), *Scab1481* (PchE) and *Scab1471* (PchF), respectively. *Sven0511* is a homologue of the proofreading type II TE *Scab1421* (PchC) and *Sven0506* is homologous to the putative salicylate synthase encoded by *scab1381*. No homologues of PchG can be found in the *S. venezuelae* genome. However, *Sven0516* is 47% similar to PchK from *P. protegens* CHA0, which has been proposed to function as a thiazoline reductase in enantiopyochelin biosynthesis.<sup>9</sup> Moreover, *Sven0508* is 48% similar to *Sven0516*,

suggesting it may also be able to function as a thiazoline reductase.

Interestingly, a homologue of *sven0515*, which encodes a putative type B radical-SAM methylase, cannot be found in any 2-hydroxyphenylthiazoline biosynthetic gene cluster reported to date. Such enzymes are known to catalyse the methylation of unactivated carbon centres in the biosynthesis of a variety of specialised metabolites.<sup>22</sup> Taken together, these analyses suggested that the *sven0503*–*sven0517* gene cluster may direct the biosynthesis of a C-methylated pyochelin derivative.

*Sven0507*/*Sven0509* and *Sven0505* are highly similar to the TetR-like *Scab1401* repressor and the AfsR family *Scab1371* activator, respectively, of pyochelin biosynthesis in *S. scabies*. Similarly, *Sven0513* and *Sven0514* are homologues of the putative ABC transporter ATPase/permease fusions encoded by *scab1431* and *scab1441*, respectively, which are likely responsible for pyochelin export. No obvious role can be postulated for the *Scab1361* homologue *Sven0503*, a putative AMP ligase, or *Sven0504*, a putative Na<sup>+</sup>/H<sup>+</sup> antiporter that is not homologous to any of the proteins encoded by the *S. scabies* pyochelin biosynthetic gene cluster.



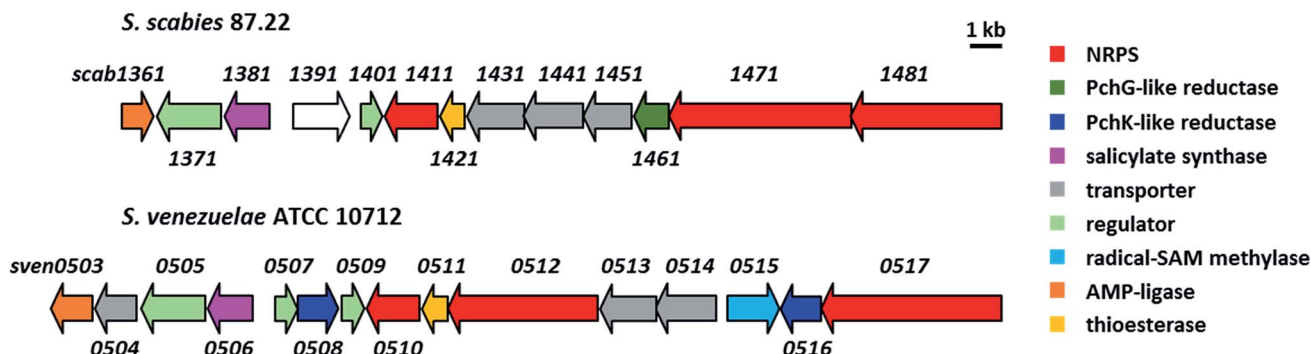


Fig. 4 Comparison of the organisation of the watasemycin and pyochelin biosynthetic gene clusters in *S. venezuelae* ATCC 10712 and *S. scabies* 87.22, respectively.

### Expression of the gene cluster in *S. coelicolor* M1152

Despite screening various growth media, we failed to detect the production of any 2-hydroxyphenylthiazoline-containing metabolites by *S. venezuelae*. This is potentially explained by the low levels of *sven0516* expression in the *bldM* mutant (Fig. 3), which is surprising given that *sven0517* is likely to be in the same operon, and possibly reflects differential mRNA stability for the two genes. We therefore elected to express the *sven0503*–*sven0517* gene cluster in the engineered host *S. coelicolor* M1152.<sup>23</sup> A clone (SV-2\_E03) from an ordered genomic cosmid library of the *S. venezuelae* chromosome containing a segment extending from *sven0496* to *sven0518* was PCR-targeted in *Escherichia coli* with a 5.2 kb *SspI* fragment from pIJ10702 that contains *oriT*, and the  $\phi$ C31 integrase gene and phage attachment site (*attP*). The resulting cosmid, SV-2\_E03::*SspI*, was introduced into *S. coelicolor* M1152 by conjugation, whereupon it integrated into the chromosomal  $\phi$ C31 *attB* site. Wild type *S. coelicolor* M1152 and the SV-2\_E03::*SspI* derivative were incubated in YD medium for five days and the culture broths were extracted with ethanol. UHPLC-ESI-Q-TOF-MS analyses identified metabolites giving rise to ions with  $m/z = 339.0840, 353.0983, 325.0675, 210.0588$  and  $224.0736$ , corresponding to  $[M + H]^+$  for the known 2-hydroxyphenylthiazolines thiazostatin 6 ( $m/z$  calculated for  $C_{15}H_{19}N_2O_3S_2^+$ : 339.0832), watasemycin 7 ( $m/z$  calculated for  $C_{16}H_{21}N_2O_3S_2^+$ : 353.0988), pyochelin 3 ( $m/z$  calculated for  $C_{14}H_{17}N_2O_3S_2^+$ : 325.0675) aerugine 4 ( $m/z$  calculated for  $C_{10}H_{12}NO_2S^+$ : 210.0583) and pulicatin A/B 8/9 ( $m/z$  calculated for  $C_{11}H_{14}NO_2S^+$ : 224.0740), respectively, in the culture extract of *S. coelicolor* M1152/SV-2\_E03::*SspI* (Fig. 5). These metabolites were not present in the culture extracts of the unmodified host. Several other liquid growth media, including TSB, ISP2 and GSP, were also found to support production of these compounds.

### Structure elucidation of metabolic products

Metabolites were isolated from the culture broths using Diaion HP-20 resin, eluting with methanol. After ethyl acetate extraction and HPLC separation, the fractions containing the compounds with molecular formulae corresponding to thiazostatin and watasemycin were collected and analysed by  $^1H$  NMR spectroscopy (see ESI<sup>†</sup>). The spectroscopic data were identical to those previously reported for thiazostatins A and B 6, and

watasemyins A and B 7, isolated from *Streptomyces toluosus* and *Streptomyces* sp. TP-A0597, respectively.

A mixture of pyochelin 3 and neopyochelin (the C-4' epimer of pyochelin) was synthesised according to a literature procedure.<sup>24</sup> Surprisingly, none of these four diastereomeric compounds had the same retention time as the metabolites with molecular formulae corresponding to pyochelin 3 (see ESI<sup>†</sup>). We thus hypothesised that these metabolites are isomers of pyochelin 3 in which C-4'' is methylated instead of the nitrogen atom of the thiazolidine. To test this hypothesis, an authentic standard of the C-4'' methylated isomer of pyochelin (hereafter referred to as isopyochelin) was synthesised as a mixture of four diastereomers *via* condensation of the known 2-hydroxyphenylthiazoline aldehyde 14 with L-2-methylcysteine (Scheme 1).

LC-MS comparisons showed that two of the synthetic isopyochelin diastereomers had the same retention time as the metabolites in the culture extract (Fig. 6). The absolute configuration of C-4'' in the natural products was established as *S* by condensing 2-hydroxyphenylthiazoline aldehyde 14 with D-2-methylcysteine and comparing the retention times of the metabolites in the extract with synthetic (4''*R*) and (4''*S*)-isopyochelins derived from L- and D-2-methylcysteine, respectively (see ESI<sup>†</sup>). Based on the assumption that natural isopyochelin and thiazostatin 6 have the same relative stereochemistry (see below), the structure of the former was assigned as 13 (Fig. 1).

### Biosynthetic role of *sven0508* and *sven0516*

The observation that the *sven0503*–*sven0517* gene cluster contains two genes encoding PchK-like putative thiazoline reductases intrigued us, because only a single thiazoline reduction appears to be involved in the biosynthesis of thiazostatin 6, watasemycin 7 and isopyochelin 13. To investigate the role played by *sven0508* and *sven0516* in the biosynthesis of 6, 7 and 13, individual in-frame deletions of these genes were created in SV-2\_E03 by PCR targeting. The mutagenized cosmids were subsequently targeted with the 5.2 kb *SspI* fragment from pIJ10702 and then introduced into *S. coelicolor* M1152 from *E. coli* ET12567/pUZ8002 by conjugation.

Ethanol extracts of culture broths from the *sven0508* and *sven0516* mutants were analysed by LC-MS. Aerugine 4, thiazostatin 6, watasemycin 7, pulicatin A/B 8/9 and





Fig. 5 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 **6**, watasemycin **7**, aerugine **4**, pulicatin A/B **8/9** and pyochelin **3**, respectively, from LC-MS analyses of the ethanol extract of *S. coelicolor* M1152/SV-2\_E03::Sspl culture broth. Two peaks are observed for thiazostatin, watasemycin and pyochelin because they exist as a mixture of two diastereomers resulting from epimerisation at C-2''.

isopyochelin **13** were all still observed in the extract from the *sven0508* mutant (see ESI<sup>†</sup>). On the other hand, none of these metabolites could be detected in the extract from the *sven0516* mutant (see ESI<sup>†</sup>). Thus we conclude that *Sven0516* plays an essential role in the biosynthesis of all the metabolic products of the *sven0503-sven0517* gene cluster.

### Revised stereochemistry of watasemycin and thiazostatin

The finding that a PchK homologue plays an essential role in assembling the metabolic products of the *sven0503-sven0517* gene cluster is in accord with our assignment of the C-4'' configuration of isopyochelin **13** as *S*. Assuming isopyochelin **13** and watasemycin **7** have the same C-4'' absolute configuration, the relative stereochemistry previously assigned to watasemycin on the basis of NOE NMR studies suggests its C-5' and C-4' stereocentres should both be *R*-configured.



Scheme 1 Synthesis of an authentic standard of (4'')*R*-isopyochelin as a mixture of stereoisomers at C-4' and C-2''.

However, the 5'*R* stereochemical assignment conflicts with the absolute stereochemistry proposed, on the basis of NOESY, Mosher's ester and CD studies, for the corresponding

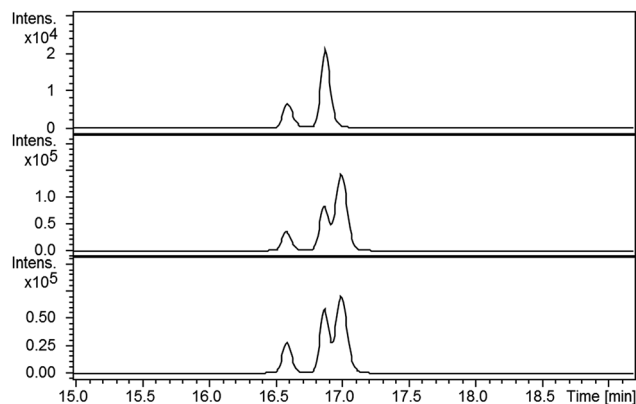


Fig. 6 Extracted ion chromatograms for  $m/z = 325.0675$ , corresponding to  $[M + H]^+$  for isopyochelin, from LC-MS analyses of the ethanol extract of *S. coelicolor* M1152/SV-2\_E03::Sspl culture broth (top), the mixture of synthetic (4'')*R*-isopyochelin diastereomers (middle) and the extract to which an approximately equimolar quantity of the synthetic standard has been added (bottom). Two peaks are observed in the top chromatogram because natural isopyochelin exists as a mixture of two diastereomers resulting from epimerisation at C-2''.



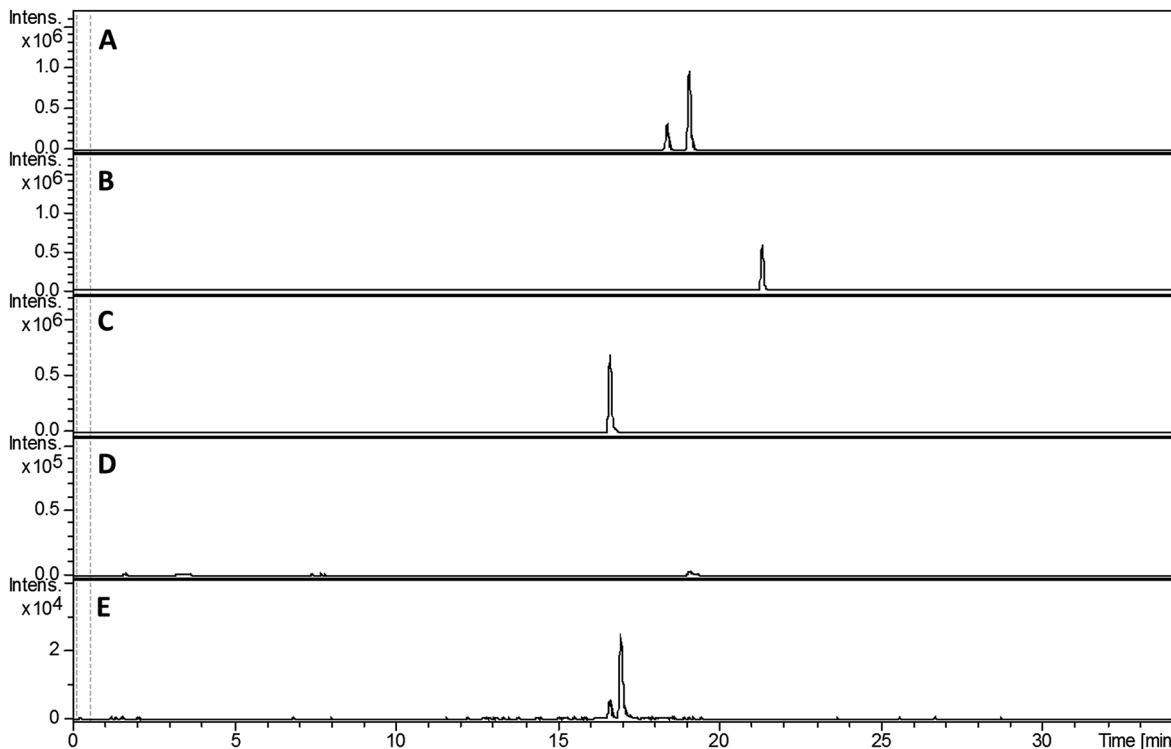


Fig. 7 Extracted ion chromatograms at  $m/z = 339.0832$  (A),  $353.0988$  (B),  $210.0583$  (C),  $224.0740$  (D) and  $325.0675$  (E), corresponding to  $[M + H]^+$  for thiazostatin, watasemycin, aerugine, pulicatin and isopyochelin, respectively, from LC-MS analyses of the ethanol extract of *S. coelicolor* M1152/SV-2\_E03::*Sspl*/ $\Delta$ *sven0515* culture broth. Two peaks are observed for thiazostatin and isopyochelin because they exist as a mixture of diastereomers resulting from epimerisation at C-2''. The peak with a retention time of approximately 21.5 minutes in the  $m/z = 353.0988$  chromatogram (B) appears, on the basis of LC-MS/MS analyses, to be due to the methyl ester of thiazostatin (see ESI<sup>†</sup>).

stereogenic centre in pulicatin A and B **8** and **9**.<sup>12</sup> The C4' hydrogen substituent and the C5' methyl group in watasemycin **7** are proposed to be *anti* to each other on the basis of NOE studies.<sup>11</sup> We thus suspect that the relative configuration of watasemycin **7** has been misassigned and hypothesize that the correct stereochemical assignment is 4'*S*, 5'*S*, 4''*S* (as shown in Fig. 1). By analogy, we propose that the stereochemistry of thiazostatin **6** is 4'*S*, 4''*S*. Although further experiments will be

required to confirm these stereochemical reassignments, it is noteworthy that the MT<sub>e</sub> domain of the PchE homologue *Sven0512* appears, on the basis of conserved domain searches,<sup>25</sup> to be non-functional. This is consistent with the 4'*S* configuration, resulting from net incorporation of L-cysteine into the thiazoline ring of thiazostatin **6**, watasemycin **7** and isopyochelin **13**, as in enantiopyochelin **5** biosynthesis (Fig. 2).<sup>9</sup>

#### Sven0515 C-methylates the thiazoline of thiazostatin

Compared with pyochelin **3**, watasemycin **7** contains two additional methyl groups at C-5' and C-4'' (Fig. 1). Based on its similarity to type B radical-SAM methylases,<sup>21</sup> *Sven0515* could be responsible for introducing one or both of these. We therefore deleted *sven0515* from SV-2\_E03, introduced the 5.2 kb *SspI* fragment from pIJ10702 and transferred the resulting construct into *S. coelicolor* M1152, as described above. LC-MS analysis of ethanol extracts from the culture broth of this strain showed that it is unable to produce watasemycin **7** or pulicatin A/B **8/9** (Fig. 7). Thus, *Sven0515* appears to be involved in the methylation of C-5', but not C-4''. To investigate the timing of C-5' methylation, we fed the metabolites in the extract of the *sven0515* mutant to the *sven0516* mutant. This resulted in complete conversion of the thiazostatin **6** in the extract to watasemycin **7** (Fig. 8), indicating that thiazostatin is the substrate of *Sven0515*.

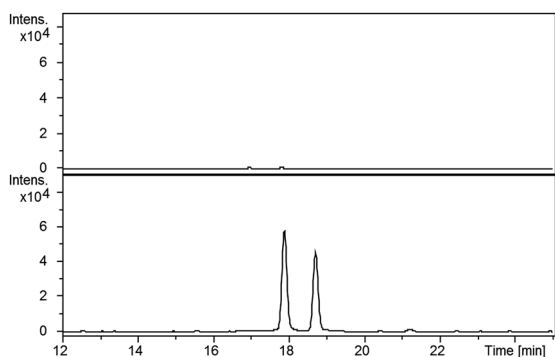


Fig. 8 Extracted ion chromatograms at  $m/z = 339.0832$ , corresponding to  $[M + H]^+$  for thiazostatin (top), and  $353.0988$ , corresponding to  $[M + H]^+$  for watasemycin, (bottom) from LC-MS analyses of the extract from the  $\Delta$ *sven0516* mutant fed with the extract from the  $\Delta$ *sven0515* mutant.



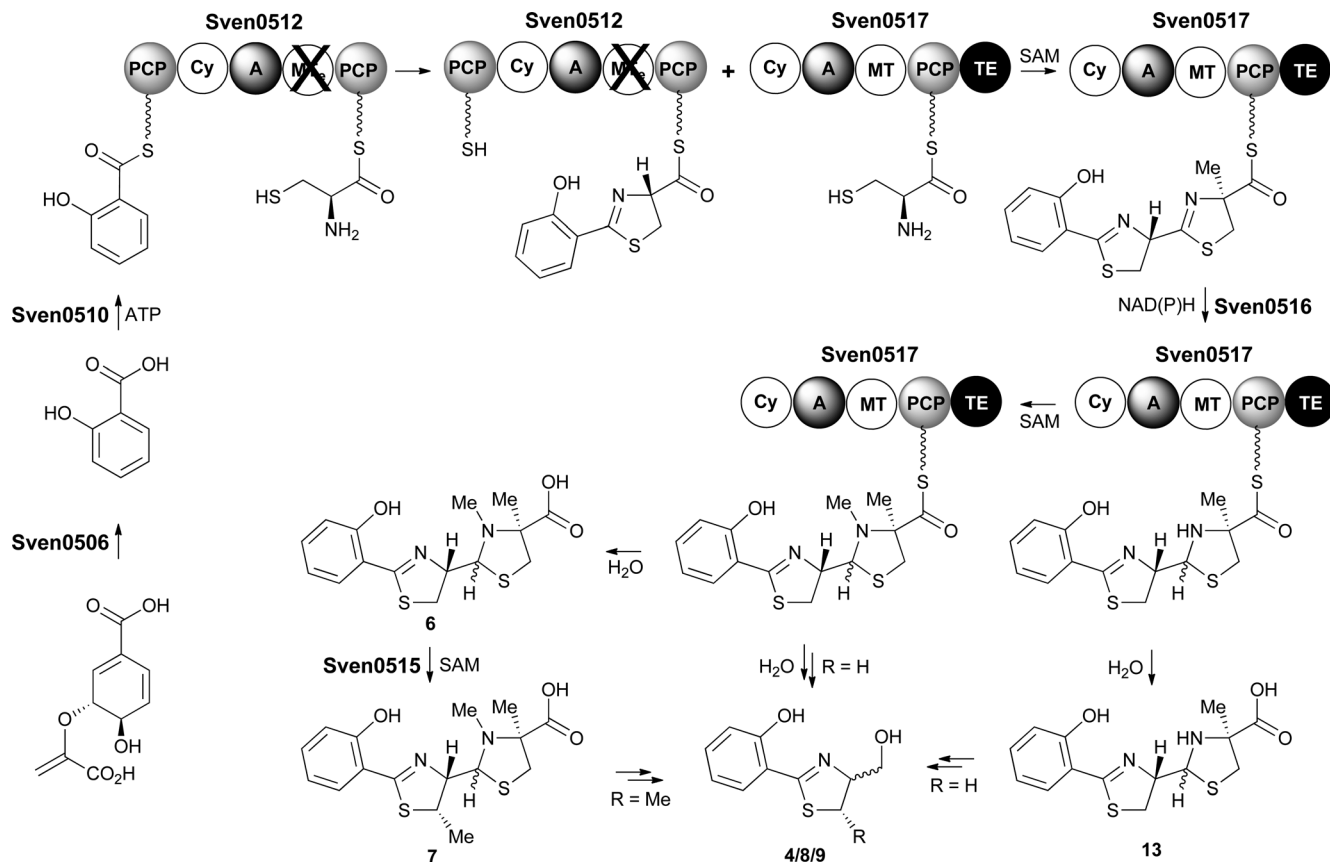


Fig. 9 Proposed pathway for the biosynthesis of thiazostatin 6, watasemycin 7, isopyochelin 13, aerugine 4 and pulicatin A/B 8/9 in *S. venezuelae*.

### Proposed pathway for watasemycin biosynthesis

Taken together, the above data lead us to propose a pathway for the biosynthesis of watasemycin 7 in *S. venezuelae* (Fig. 9). The early stages of this pathway, up to the formation of the 2-hydroxyphenyl-bis-thiazolinyl thioester intermediate attached to the PCP domain of Sven0517, mirror the biosynthesis of enantiopyochelin 5 (Fig. 2). At this point the MT domain of Sven0517 appears to catalyse methylation of C-4'' (Fig. 9). An analogous transformation has been shown to occur in the biosynthesis of yersiniabactin.<sup>26</sup> Sven0516 catalyses thiazoline reduction and the MT domain of Sven0517 then methylates the nitrogen atom of the resulting thiazolidine, as in pyochelin biosynthesis.<sup>16</sup> Hydrolysis of the thioester by the TE domain affords thiazostatin 6, which is methylated at C-5' by Sven0515 to give watasemycin 7. Isopyochelin 13 presumably results from TE-catalysed thioester hydrolysis prior to the *N*-methylation reaction, suggesting that this may be a slow step in thiazostatin 6 biosynthesis. The biosynthetic origins of aerugine 4 and pulicatin A/B 8/9 are unclear, but it seems likely that they arise from hydrolytic cleavage and subsequent reduction of thiazostatin 6/isopyochelin 13 and watasemycin 7, respectively.

## Conclusions

We have identified the known *Streptomyces* metabolites aerugine 4, thiazostatin 6, watasemycin 7 and pulicatin A/B 8/9,

and the novel natural product isopyochelin 13 as the metabolic products of a cryptic 2-hydroxyphenylthiazoline biosynthetic gene cluster in *S. venezuelae* ATCC10712 using a heterologous expression approach. The absolute stereochemistry of isopyochelin 13 was assigned as 4''*S* by comparison with synthetic standards. In combination with the absolute and relative stereochemistry reported for pulicatin A and B 8 and 9,<sup>12</sup> previous investigations of the relative stereochemistry of watasemycin 7,<sup>11</sup> and biosynthetic considerations, this prompted us to assign the stereochemistry of watasemycin 7 and thiazostatin 6 as 4'*S*, 5'*S*, 4''*S* and 4'*S*, 4''*S*, respectively. Gene deletion experiments established an essential role for the PchK homologue Sven0516 in the biosynthesis of all the metabolic products of the gene cluster and showed that the type B radical-SAM methylase homologue encoded by *sven0515* is responsible for the conversion of thiazostatin 6 to watasemycin 7. Type B radical-SAM methylases are known to catalyse methylation of unactivated carbon centres in the biosynthesis of several different classes of natural products, including aminoglycosides,  $\beta$ -lactams, phosphonates, and ribosomally biosynthesised and post-translationally-modified peptides.<sup>22</sup> However, to the best of our knowledge the Sven0515-catalysed methylation of thiazostatin 6 is the first experimentally-validated example of such a reaction in the biosynthesis of a nonribosomal peptide.





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