

Identification of AstG1, A LAL Family Regulator that Positively Controls Ansatrienins Production in *Streptomyces* sp. XZQH13

Chao Xie · Jing-Jing Deng · Hao-Xin Wang

Received: 19 December 2014 / Accepted: 26 January 2015 / Published online: 18 March 2015
© Springer Science+Business Media New York 2015

Abstract Ansamycins is a group of type I polyketides characterized by the unique starter unit 3-amino-5-hydroxybenzoic acid. This family of secondary metabolites shows diverse biological activities, well-known members of which include rifamycin, geldanamycin, and maytansine. Previously, we isolated an AHBA synthase gene-positive strain *Streptomyces* sp. XZQH13 containing a “silent” ansamycin biosynthetic gene cluster *ast*. The constitutive expression of the Large-ATP-binding regulators of the LuxR family regulator gene *astG1* located within the cluster triggered the expression of the biosynthetic genes. Reverse transcription-PCR experiments showed that the expression of the key biosynthetic genes, *astB4*, *astD1*, and *astF1*, was induced in the *astG1* over-expression mutant compared to the wild type. This led to the isolation of two known ansatrienins, hydroxymycotrienin A (**1**) and thiazinotrienomycin G (**2**), which were identified by analysis of the mass spectral and NMR spectral data, from the mutant. These observations suggest

that *astG1* is probably a pathway-specific positive regulator for the biosynthesis of ansatrienin.

Introduction

Streptomyces are prolific producers of pharmaceutically useful secondary metabolites with plenty of biological activities, such as antibiotic, immunosuppressive, and anti-tumor activities. Genome sequencing shows that each *Streptomyces* strain contains 20 or more potential secondary metabolites biosynthetic gene clusters [7, 14], the majority of which are “silent” or expressed in an extremely low level under laboratory culture conditions. Strategies to activate these clusters have been developed and utilized to produce novel natural products [3, 13]. For example, overexpression or deletion of pathway-specific regulatory genes may induce the expression of cryptic biosynthetic gene clusters to a detectable level [1, 8]. LuxR-type proteins are unique members of the TetR superfamily of transcription factors, which are widely distributed among bacteria and share a conserved helix-turn-helix (HTH) DNA binding motif in their N-terminal domains [16]. LuxR proteins function as dimers to recognize and bind DNA sequences to control genes that are involved in quorum sensing, biosynthesis, and metabolism. LAL can directly and indirectly controls the expression of multiple genes and bind to multiple binding sites in its target promoters, allowing for positively and negatively control large regulons of genes [19]. Constitutive expression of LuxR family regulator has been proved to be a useful approach to activate “silent” biosynthetic gene clusters [11, 12, 21].

Ansamycins is a family of type I polyketides, which uses AHBA (3-amino-5-hydroxybenzoic acid) as its unique

Chao Xie and Jing-Jing Deng have contributed equally to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-015-0798-6) contains supplementary material, which is available to authorized users.

C. Xie · H.-X. Wang (✉)
State Key Laboratory of Microbial Technology, School of Life Science, Shandong University, Jinan 250100,
People’s Republic of China
e-mail: wanghaoxin@sdu.edu.cn

J.-J. Deng
Key Laboratory of Chemical Biology (Ministry of Education),
School of Pharmaceutical Sciences, Shandong University,
Jinan 250012, Shandong, People’s Republic of China

starter unit and amide synthase to release the polyketide chain [9]. One representative group of ansamycins is ansatrienins. Ansatrienins possess a 1,4-quinone or a 1,4-hydroquinone moiety as chromophore and a triene moiety, and exhibit various biological and medical activities, such as antibacterial, antifungal, antitumor, and cytotoxic activities [18]. Although a partial ansatrienin biosynthetic gene cluster *ans* in *Streptomyces collinus* and the entire one *myc* in *Streptomyces flaveolus* have been identified [2, 15], the regulation of ansatrienin biosynthesis have not been reported.

Streptomyces sp. XZQH13 is an AHBA synthase gene-positive strain, which was isolated from a soil sample collected from Qinghai, China [20]. However, no ansamycins were obtained by large-scale fermentation under different culture conditions, which indicated that the potential ansamycin gene cluster is “silent” or weakly expressed under standard laboratory conditions. Here we report our successful activation of this cryptic cluster by constitutive expression of the LAL family regulator gene *astG1* located in *ast* cluster. As a result, two known ansatrienins, identified as hydroxymycotrienins A (**1**) and thiazinotrienomycin G (**2**), were isolated from the mutant. In addition, the upregulation of the transcription of the key biosynthetic genes was observed in the mutant by RT-PCR experiments. To the best of our knowledge, this is the first report that a “silent” ansamycin biosynthetic cluster can be activated by constitutive expression of a LAL family regulator.

Materials and Methods

Strains, Media and Culture Conditions

Escherichia coli (*E. coli*) strain XL-1 Blue (Stratagene) was used as hosts for DNA cloning and sequencing. *E. coli* ET12567/pUZ8002 was used to transfer DNA into *Streptomyces* sp. XZQH13 by conjugation. The *Streptomyces* strains were cultured at 30 °C on SFM solid medium (1.5 % agar, 2 % D-mannitol, 2 % soybean meal, pH 7.2) for conjugation between *E. coli* and *Streptomyces*, or in ISP2 liquid medium (yeast extract 4 g, malt extract 10 g, glucose 4 g, 1 L ddH₂O, pH 7.2–7.4) for mycelial growth, or on YMG solid medium (4 % yeast extract, 10 % malt extract, 4 % glucose, pH 7.2) for fermentation. All *E. coli* strains were cultivated in Luria–Bertani (LB) or on LB agar plates at 37 °C. General approaches for *E. coli* or *Streptomyces* manipulations were performed according to standard methods [10, 17]. The final antibiotic concentrations are: ampicillin (100 µg/ml), nalidixic acid (25 µg/ml), and apramycin (30 µg/ml).

Bioinformatic Analysis

The ORFs were deduced from the sequence with the FramePlot 4.0beta program (<http://nocardia.nih.gov/fp4/>). The function of putative proteins were deduced via homology-based analysis using the BLAST online program (<http://www.ncbi.nlm.nih.gov/blast/>).

Constitutive Expression of the LAL Family Regulator Gene *astG1*

The *astG1* gene was amplified from fosmid 17-10F with primer pairs of QH13-LAL-F and QH13-LAL-R (Table S1). The PCR product was ligated into pMD18-T vector (Takara) and sequenced. The NdeI/XbaI fragment was then inserted downstream of *ermE***p* into pJTU824 vector to give pJTU824-*astG1* [22]. The resulting plasmid pJTU824-*astG1* was transformed into *E. coli* strain ET12567/pUZ8002, which was then mated with strain XZQH13 for conjugal transfer of the vector. The mutants were selected on SFM medium plates to produce XZQH13/pJTU824-*astG1*. Meanwhile, the control strain XZQH13/pJTU824 was also constructed by conjugation of the pJTU824 vector into strain XZQH13. The conjugants were inoculated into 2 ml ISP2 liquid medium and cultivated for 3 days at 30 °C, 240 rpm. The mycelia were collected for total DNA extraction followed by PCR verification with primer pairs of M13-47 and QH13-LAL-R.

Reverse Transcription-PCR Analysis

Spores of XZQH13/pJTU824 and XZQH13/pJTU824-*astG1* were collected from ISP2 agar plates and stored in 10 % glycerol aqueous solution under –80 °C. Then the spores were inoculated into 50 ml ISP2 liquid medium in 250 ml erlenmeyer flasks with spring, and incubated for 72 h at 30 °C, 300 rpm. The resulting liquid cultures were spread onto cellophane disks on ISP2 plates and cultivated at 30 °C. Mycelia were harvested after 3 and 6 days, respectively. Total RNA was isolated using RNAiso Plus Kit (Takara) and reverse transcription was conducted using PrimeScript II 1st Strand cDNA Synthesis Kit (Takara). Primers for AHBA synthase gene (*astB4*), A-domain of the first PKS gene (*astD1*), and a proposed post-PKS modification gene (*astF1*) (Table S1) were designed and tested using chromosomal DNA as a template. The housekeeping gene *hrdB* was chosen as the internal control.

Production and Analysis of the Metabolites in XZQH13/pJTU824-*astG1*

XZQH13/pJTU824 and XZQH13/pJTU824-*astG1* strains were grown on solid ISP2 medium for 12 days at 30 °C. To

extract the metabolites, the 40 ml culture was collected and extracted with EtOAc/MeOH/AcOH (85/15/5, v/v/v) at room temperature overnight. The organic phase was dried with a rotavapor (R-3; Buchi) to obtain the crude extract. The extract was dissolved in 2 ml methanol. A 20- μ l aliquot of each extract was analyzed by high-pressure liquid chromatography (HPLC; Agilent Technologies 1260 infinity) using a reverse-phase column (ZORBAX Eclipse SB-C₁₈ 5 μ m, column ID: 4.6 \times 250 mm, flow rate: 1 ml/min, UV detection at 274 nm). Water-0.035 % TFA (solvent A) and CH₃CN-0.035 % TFA (solvent B) were used as the mobile phases. The HPLC program was as follows: 5–35 % solvent B in solvent A for the first 0–5 min, with an increase to 55 % B at 19 min, to 65 % B at 20 min, to 100 % B in 3 min, followed by 4 min with 100 % B, and back to 5 % B at 28, followed by 2 min with 5 % B. The metabolites were detected at 274 nm on a UV detector. The main extra absorption peaks appear at about 25 and 26 min, respectively.

Preparation of Ansatrienins and MS and NMR Analysis

The XZQH13/pJTU824-*astG1* mutant was incubated on solid ISP2 medium (10 l) for 12 days at 30 °C. The cultured agar was extracted three times with EtOAc/MeOH/AcOH (85:15:5, v/v/v) at room temperature overnight and partitioned between water and EtOAc. Then, the EtOAc extract was dried with Na₂SO₄. The solvent was removed under vacuum, and the residue was further partitioned between petroleum ether and MeOH (1:1, v/v). The combined MeOH layers were evaporated *in vacuo*. The MeOH extract (2.06 g) was subjected to Sephadex LH-20 eluted with MeOH to obtain nine fractions: Fr.1-Fr.9. Fr.4 was further subjected to medium-pressure liquid chromatography (MPLC, RP-18 silica gel, 30 g), five fractions (Fr.4a-Fr.4e) were obtained from the elution of 50, 70, 80, 90, and 100 % MeOH in water, respectively, 200 ml for each gradient. Fr.4b was subjected to MPLC over the RP-18 silica gel (30 g), four fractions (Fr.4b1-Fr.4b4) were obtained from the elution of 60, 65, 70, and 100 % MeOH in water, respectively, 100 ml for each gradient. Fr.4b2 was finally purified by semi-preparative reverse-phase HPLC (ZORBAX Eclipse XDB-C₁₈ 5 μ m, column ID: 9.4 \times 250 mm, flow rate: 4 ml/min, elution: CH₃CN/H₂O (50:50, v/v), UV detection at 274 nm) to yield **2** (5 mg). Fr.4b3 was purified by semi-preparative reverse-phase HPLC (ZORBAX Eclipse XDB-C₁₈ 5 μ m, column ID: 9.4 \times 250 mm, flow rate: 4 ml/min, elution: CH₃CN/H₂O (35:65, v/v), UV detection at 274 nm) to yield **1** (3 mg). To analyze the structures of the compounds, HR-ESI-MS data were carried out on an LTQ-Orbitrap XL mass spectrometer. NMR spectra (¹H and ¹³C-NMR) were measured on Bruker DRX-600 MHz NMR spectrometer (Bruker Daltonics Inc., Billerica, Massachusetts) at 600/150 MHz, respectively, in CD₃OD, δ in ppm relative to (CH₃)₄Si.

Results and Discussion

Sequence Analysis of Partial *ast* Gene Cluster

Previously, a partial AHBA synthase gene (*astB4*) fragment was amplified from strain XZQH13 and sequenced. In general, the AHBA synthase genes with close phylogenetic relationships were believed to be involved in the biosynthesis of similar or identical polyketide backbones [6, 20]. To predict the structure of the products encoding by the *ast* cluster, a neighbor-joining tree was built for the AHBA synthase (AstG1) in *ast* cluster with homologous ones in other ansamycin biosynthetic gene clusters (Fig. 1a). The cladogram reveals that AstG1 is closest to MycB4 from *Streptomyces flaveolus* and AnsF from *Streptomyces collinus*, which have been identified to be involved in ansatrienins biosynthesis [2, 15]. In addition, AstG1 shows amino acid identities of 89 and 87 % with MycB4 and AnsF, respectively, which indicated that the *astB4* containing gene cluster has the potential to synthesis new ansatrienin analogs according to our experience.

Since no ansamycins were isolated from strain XZQH13 by large-scale fermentation under different culture conditions, the potential ansamycin gene cluster was assumed to be “silent”. In order to activate this gene cluster, we conducted a genome survey of strain XZQH13. Bioinformatic analysis revealed only one putative ansamycin gene cluster *ast* (Table S2, GenBank accession number: KF813023.1 and KP284551), in which a subset of AHBA biosynthetic genes and the amide synthase gene required for the release and cyclization of the polyketide chain are presented. Since we only performed genome survey sequencing of strain XZQH13 to obtain 100 \times coverage of the genome, the polyketide synthase (PKS) genes cannot be completely assembled due to highly repeated DNA sequences within the PKS genes. The gene organization of *ast* was similar to the reported ansatrienin biosynthetic cluster *myc* of *Streptomyces flaveolus*, [15] excepting that extra PKS and thioesterase genes were presented in *ast* and several genes were absent compared to *myc* (Fig. 1b).

Activation of the *ast* Gene Cluster

To further confirm that no ansamycins has been identified was due to the poor or no expression of the *ast* cluster, we examined the expression of selected biosynthetic genes within the cluster, including the AHBA synthase gene (*astB4*), the first PKS gene (*astD1*), and the post-PKS modification gene (*astF1*), under two growth conditions. As predicted, RT-PCR analysis indicated that these genes were expressed very poorly or not at all throughout growth (Fig. 2).

The analysis of the gene cluster identified *astG1* gene encoding a putative positive regulator of the LAL family

Fig. 1 The biosynthetic genes of ansatrienins in *Streptomyces* XZQH13. **a** Phylogenetic analysis of AHBA synthases. Similar sequences were aligned with ClustalW, and the tree shown was generated using the MEGA 5.05 software. **b** The ansatrienin biosynthetic gene clusters (*ast* and *myc*) from strain XZQH13 and *Streptomyces flaveolus* DSM 9954, respectively

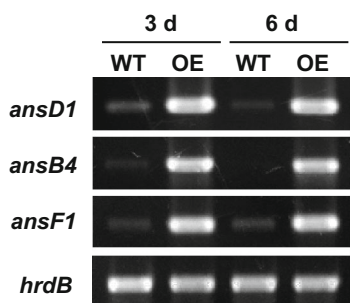
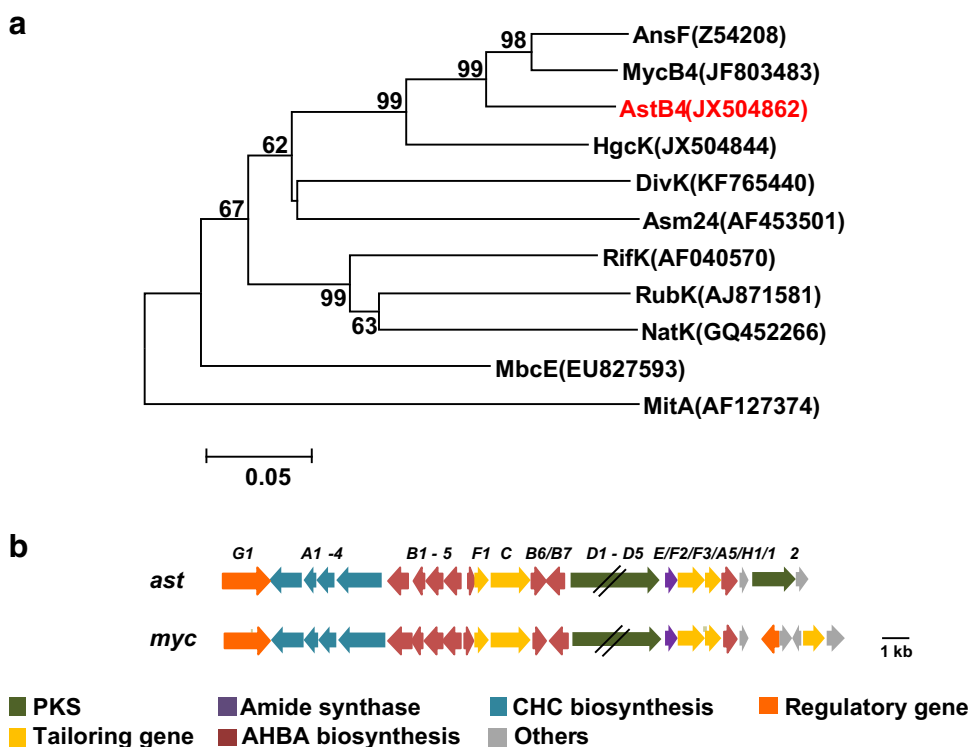


Fig. 2 AstG1 activated the expression of the *ast* gene. RT-PCR analysis of the ansatrienin biosynthetic genes in XZQH13 wild type (WT) and the *astG1* overexpression mutant XZQH13/pJTU824-*astG1* (OE). *hrdB* was selected as positive control

protein. Members of the LAL family have been previously described as activators of polyketide biosynthesis in actinomycetes [11, 12, 21]. Thus, we decided to examine whether constitutive expression of *astG1* can lead to activation of the *ast* gene cluster. The *astG1* gene was cloned into the conjugative and integrative vector pJTU824, placing it under the control of the *ermE** promoter. The resulting construct was integrated into the chromosome of strain XZQH13 to create XZQH13/pJTU824-*astG1*, and the control strain XZQH13/pJTU824 containing the empty pJTU824 vector was also created. Comparative transcriptional analyses by RT-PCR showed that the selected genes within the *ast* cluster were expressed in the XZQH13/pJTU824-*astG1* mutant throughout growth, suggesting that

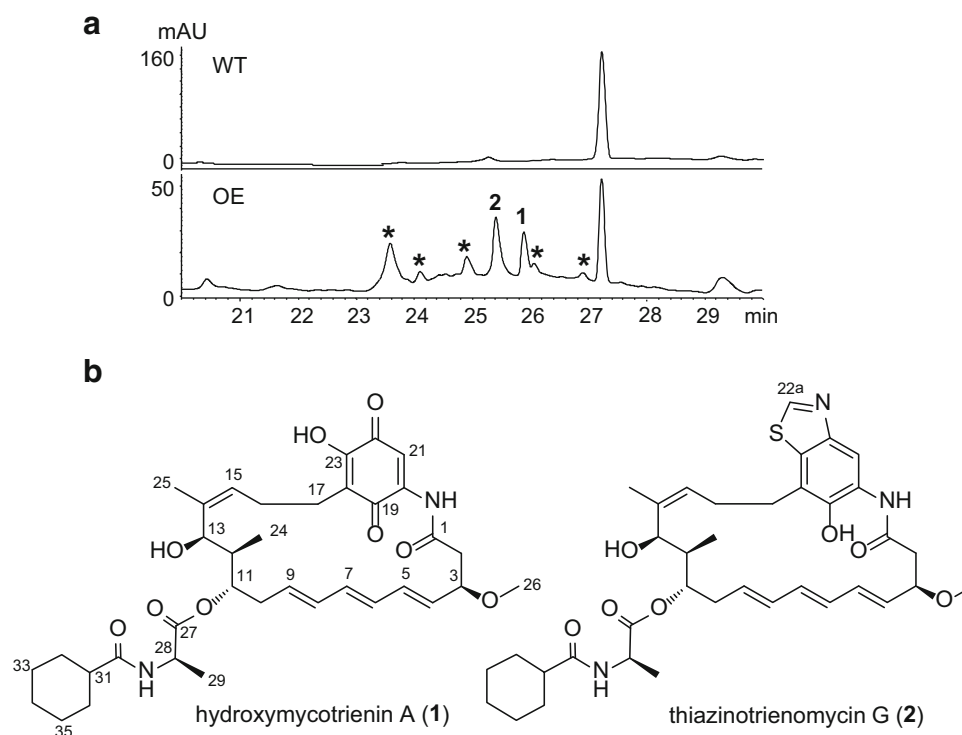
the *astG1* is probably a pathway-specific activator of the *ast* cluster (Fig. 2).

Isolation and Structure Elucidation of the Metabolic Products of the *ast* Cluster

As the *ast* gene cluster seemed to be induced in XZQH13/pJTU824-*astG1*, we sought to monitor the production of new metabolites in the mutant. The metabolites of strains XZQH13/pJTU824-*astG1* and XZQH13/pJTU824 were analyzed by comparative metabolic profiling using HPLC. Thus, a series of extra absorption peaks were detected in XZQH13/pJTU824-*astG1* mutant (Fig. 3a). Large-scale cultivation was carried out to provide sufficient amounts for the isolation of the two major products and full structure elucidation. The structures of compounds **1** and **2** were elucidated by the analysis of their ESI-MS and NMR spectroscopic data. (Figure 3b, Table S3 and Figs. S1–S6). The comparison of these data to those in the literature readily revealed that compounds **1** and **2** are identical to hydroxymycotrienin A [5] and thiazinotrienomycin G [4], respectively. These results indicated that the *ast* gene cluster is responsible for the biosynthesis of ansatrienins in strain XZQH13, which is further supported by the fact that only one ansamycin gene cluster, *ast*, is identified in the genome of strain XZQH13.

Previously, Liu et al. had proposed a biosynthetic pathway for mycotrienins in *Streptomyces flaveolus* [15]. The

Fig. 3 AstG1 activated the production of ansatrienins in *Streptomyces* sp. XZQH13. **a** HPLC analysis of XZQH13 wild type (WT) and the *astG1* overexpression mutant XZQH13/pJTU824-*astG1* (OE). Asterisk uncharacterized ansatrienin analogs in OE. **b** Chemical structures of hydroxymycotrienin A (1) and thiazinotrienomycin G (2)



formation of the core structure of ansatrienins in strain XZQH13 is proposed to be the same as readily suggested for mycotrienins in *S. flaveolus*. The PKSs utilize AHBA as the starter unit, and elongated the polyketide chain by incorporation of two molecules of methylmalonyl-CoA and six molecules of malonyl-CoA, and then the mature polyketide chain is transferred to the amide synthase, which catalyzes the release and cyclization of the polyketide chain to generate proansatrienins. For the post-PKS modifications, the nonribosomal peptide synthetase (NRPS) MycC and an esterase MycF4 have been proposed to be involved in incorporating a D-Ala residue at C-11. However, the homologue of *mycF4* gene was absent in *ast* gene cluster, which suggested that *mycF4* may be not essential for the formation of the D-Ala ester in ansatrienins. Still, we cannot exclude the possibility that the *mycF4* analogue was located outside the partial identified *ast* gene cluster. The MycF1's homolog AstF1 is proposed to be responsible for the attachment of the cyclohexanecarboxylic acid moiety.

Conclusions

In summary, we have identified a “silent” ansatrienin gene cluster in *Streptomyces* sp. XZQH13 and successfully activated it by constitutive expression of the LAL family regulator gene *astG1*, as evidenced by the induction of expression of the key biosynthetic genes relative to the control and isolation of two ansatrienins from the mutant. This indicated that AstG1 is

probably a pathway-specific activator for the biosynthesis of ansatrienins. The constitutive expression of *astG1* analogs presented in other ansatrienin producing strains has the potential to increase the yield of ansatrienins and facilitate the isolation of new derivatives with improved biological activities. The finding reported here again highlights the power of manipulation of the pathway-specific regulators in genome mining of the cryptic biosynthetic pathways identified in actinomycete.

Acknowledgments This study was supported by the National Natural Science Foundation of China (31100079), 973 Programs (2012CB721005; 2010CB833802), and Program for Changjiang Scholars and Innovative Research Team in University (IRT13028).

References

- Bunet R, Song L, Mendes MV, Corre C, Hotel L, Rouhier N, Framboisier X, Leblond P, Challis GL, Aigle B (2011) Characterization and manipulation of the pathway-specific late regulator AlpW reveals *Streptomyces ambofaciens* as a new producer of Kinamycins. *J Bacteriol* 193(5):1142–1153. doi:10.1128/JB.01269-10
- Chen S, von Bamberg D, Hale V, Breuer M, Hardt B, Muller R, Floss HG, Reynolds KA, Leistner E (1999) Biosynthesis of ansatrienin (mycotrienin) and naphthomycin. Identification and analysis of two separate biosynthetic gene clusters in *Streptomyces collinus* Tu 1892. *Eur J Biochem* 261(1):98–107. doi:10.1046/j.1432-1327.1999.00244.x
- Chiang YM, Chang SL, Oakley BR, Wang CC (2011) Recent advances in awakening silent biosynthetic gene clusters and linking orphan clusters to natural products in microorganisms.

- Curr Opin Chem Biol 15(1):137–143. doi:[10.1016/j.cbpa.2010.10.011](https://doi.org/10.1016/j.cbpa.2010.10.011)
4. Hosokawa N, Naganawa H, Hamada M, Iinuma H, Takeuchi T, Tsuchiya KS, Hori M (2000) New triene-ansamycins, thiazinotrienomycins F and G and a diene-ansamycin, benzoxazomycin. J Antibiot (Tokyo) 53(9):886–894. doi:[10.7164/antibiotics.53.886](https://doi.org/10.7164/antibiotics.53.886)
 5. Hosokawa N, Naganawa H, Hamada M, Takeuchi T, Ikeno S, Hori M (1996) Hydroxymycotrienins A and B, new ansamycin group antibiotics. J Antibiot (Tokyo) 49(5):425–431. doi:[10.7164/antibiotics.49.425](https://doi.org/10.7164/antibiotics.49.425)
 6. Huitu Z, Linzhuan W, Aiming L, Guizhi S, Feng H, Qiuping L, Yuzhen W, Huanzhang X, Qunjie G, Yiguang W (2009) PCR screening of 3-amino-5-hydroxybenzoic acid synthase gene leads to identification of ansamycins and AHBA-related antibiotic producers in Actinomycetes. J Appl Microbiol. doi:[10.1111/j.1365-2672.2008.04010.x](https://doi.org/10.1111/j.1365-2672.2008.04010.x)
 7. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S (2003) Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat Biotechnol 21(5):526–531. doi:[10.1038/nbt820](https://doi.org/10.1038/nbt820)
 8. Kallifidas D, Kang HS, Brady SF (2012) Tetarimycin A, an MRSA-active antibiotic identified through induced expression of environmental DNA gene clusters. J Am Chem Soc 134(48):19552–19555. doi:[10.1021/ja3093828](https://doi.org/10.1021/ja3093828)
 9. Kibby JJ, McDonald IA, Rickards RW (1980) 3-Amino-5-hydroxybenzoic acid as a key intermediate in ansamycin and maytansinoid biosynthesis. J Chem Soc Chem Commun 16:768–769. doi:[10.1039/C39800000768](https://doi.org/10.1039/C39800000768)
 10. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical streptomyces genetics. The John Innes Foundation, Norwich
 11. Kuscer E, Coates N, Challis I, Gregory M, Wilkinson B, Sheridan R, Petkovic H (2007) Roles of rapH and rapG in positive regulation of rapamycin biosynthesis in *Streptomyces hygrosopicus*. J Bacteriol 189(13):4756–4763. doi:[10.1128/JB.00129-07](https://doi.org/10.1128/JB.00129-07)
 12. Laureti L, Song L, Huang S, Corre C, Leblond P, Challis GL, Aigle B (2011) Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens*. Proc Natl Acad Sci USA 108(15):6258–6263. doi:[10.1073/pnas.1019077108](https://doi.org/10.1073/pnas.1019077108)
 13. Ochi K, Hosaka T (2013) New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. Appl Microbiol Biotechnol 97(1):87–98. doi:[10.1007/s00253-012-4551-9](https://doi.org/10.1007/s00253-012-4551-9)
 14. Omura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, Takahashi Y, Horikawa H, Nakazawa H, Osonoe T, Kikuchi H, Shiba T, Sakaki Y, Hattori M (2001) Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. Proc Natl Acad Sci USA 98(21):12215–12220. doi:[10.1073/pnas.211433198](https://doi.org/10.1073/pnas.211433198)
 15. Qu XD, Lei C, Liu W (2011) Transcriptome mining of active biosynthetic pathways and their associated products in *Streptomyces flaveolus*. Angew Chem Int Ed 50(41):9651–9654. doi:[10.1002/anie.201103085](https://doi.org/10.1002/anie.201103085)
 16. Ramos JL, Martinez-Bueno M, Molina-Henares AJ, Teran W, Watanabe K, Zhang X, Gallegos MT, Brennan R, Tobes R (2005) The TetR family of transcriptional repressors. Microbiol Mol Biol Rev 69(2):326–356. doi:[10.1128/MMBR.69.2.326-356.2005](https://doi.org/10.1128/MMBR.69.2.326-356.2005)
 17. Sambrook J, Russell DW, Russell DW (2001) Molecular cloning: a laboratory manual (3-volume set)
 18. Sugita M, Sasaki T, Furihata K, Seto H, Otake N (1982) Studies on mycotrienin antibiotics, a novel class of ansamycins. II. Structure elucidation and biosynthesis of mycotrienins I and II. J Antibiot (Tokyo) 35(11):1467–1473. doi:[10.7164/antibiotics.35.1460](https://doi.org/10.7164/antibiotics.35.1460)
 19. van Kessel JC, Ulrich LE, Zhulin IB, Bassler BL (2013) Analysis of activator and repressor functions reveals the requirements for transcriptional control by LuxR, the master regulator of quorum sensing in *Vibrio harveyi*. MBio 4(4):e00378. doi:[10.1128/mBio.00378-13e00378-13](https://doi.org/10.1128/mBio.00378-13e00378-13)
 20. Wang H, Chen Y, Ge L, Fang T, Meng J, Liu Z, Fang X, Ni S, Lin C, Wu Y, Wang M, Shi N, He H, Hong K, Shen Y (2013) PCR screening reveals considerable unexploited biosynthetic potential of ansamycins and a mysterious family of AHBA-containing natural products in actinomycetes. J Appl Microbiol 115(1):77–85. doi:[10.1111/jam.12217](https://doi.org/10.1111/jam.12217)
 21. Wilson DJ, Xue YQ, Reynolds KA, Sherman DH (2001) Characterization and analysis of the PikD regulatory factor in the pikromycin biosynthetic pathway of *Streptomyces venezuelae*. J Bacteriol 183(11):3468–3475. doi:[10.1128/jb.183.11.3468-3475.2001](https://doi.org/10.1128/jb.183.11.3468-3475.2001)
 22. Wu YY, Kang QJ, Shen YM, Su WJ, Bai LQ (2011) Cloning and functional analysis of the naphthomycin biosynthetic gene cluster in *Streptomyces* sp. CS. Mol Biosyst 7(8):2459–2469. doi:[10.1039/c1mb05036b](https://doi.org/10.1039/c1mb05036b)