3 Biotech (2017) 7:250
DOI 10.1007/s13205-017-0875-6

REVIEW ARTICLE

Regulatory genes and their roles for improvement of antibiotic biosynthesis in *Streptomyces*

Fengjuan Lu[1] · Yanyan Hou[1] · Heming Zhang[1] · Yiwen Chu[2] · Haiyang Xia[3] · Yongqiang Tian[1]

Received: 20 April 2017 / Accepted: 7 July 2017 / Published online: 17 July 2017

Abstract The numerous secondary metabolites in *Streptomyces* spp. are crucial for various applications. For example, cephamycin C is used as an antibiotic, and avermectin is used as an insecticide. Specifically, antibiotic yield is closely related to many factors, such as the external environment, nutrition (including nitrogen and carbon sources), biosynthetic efficiency and the regulatory mechanisms in producing strains. There are various types of regulatory genes that work in different ways, such as pleiotropic (or global) regulatory genes, cluster-situated regulators, which are also called pathway-specific regulatory genes, and many other regulators. The study of regulatory genes that influence antibiotic biosynthesis in *Streptomyces* spp. not only provides a theoretical basis for antibiotic biosynthesis in *Streptomyces* but also helps to increase the yield of antibiotics via molecular manipulation of these regulatory genes. Currently, more and more emphasis is being placed on the regulatory genes of antibiotic biosynthetic gene clusters in *Streptomyces* spp., and many studies on these genes have been performed to improve the yield of antibiotics in *Streptomyces*. This paper lists many antibiotic biosynthesis regulatory genes in *Streptomyces* spp. and focuses on frequently investigated regulatory genes that are involved in pathway-specific regulation and pleiotropic regulation and their applications in genetic engineering.

Keywords Antibiotic biosynthetic gene cluster · Regulatory gene · Regulatory mechanism · *Streptomyces* · Secondary metabolites

✉ Yongqiang Tian
yqtian@scu.edu.cn

[1] Key Laboratory of Leather Chemistry and Engineering (Sichuan University), Ministry of Education and College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu, Sichuan, China

[2] Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu, Sichuan, China

[3] Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Introduction

Secondary metabolites of *Streptomyces*, including antibiotics, immunomodulators, enzyme inhibitors and other bioactive substances, often have significant medicinal value. However, wild strains usually produce low levels of antibiotics. There is a large and complicated regulatory network in many *Streptomyces* strains, and the biosynthesis of one antibiotic in one strain may be controlled by more than one regulatory mechanism. For example, the production of actinorhodin (ACT) in *Streptomyces coelicolor* is regulated by both the cluster-situated regulator (CSR) *actII-ORF4* (Fernández-Moreno et al. 1991) and the pleiotropic regulatory gene *cprB* (Onaka et al. 1998). Further, there is also more than one regulatory gene that affects the biosynthesis of a single antibiotic. For example, *dnrI*, *dnrO* and *dnrN* (Kitani et al. 2008; Parajuli et al. 2005) all regulate the production of daunorubicin (DNR) in *Streptomyces peucetius*.

To better and more comprehensively understand the mechanisms of antibiotic biosynthesis regulation, in this paper, we classify the antibiotic biosynthetic regulators in various *Streptomyces* strains that are associated with antibiotic production into three types. This work will provide a theoretical basis for the molecular perturbation of regulatory genes and will help with manipulating the

antibiotic biosynthetic pathways and accordingly improving antibiotic production.

Regulatory genes involved in pathway-specific regulation

The CSRs, located within the antibiotic biosynthetic clusters, can modulate the antibiotic biosynthetic genes of the clusters in which they are included (Martín and Liras 2010; Rodríguez et al. 2013). The CSRs can only affect the biosynthetic pathway of a single, specific antibiotic and act as a master switch for biosynthesis of that individual antibiotic; this regulation is called pathway-specific regulation (Bibb 1996; Novakova et al. 2011). Some CSRs encode proteins that belong to a family known as the *Streptomyces* antibiotic regulatory proteins (SARPs). SARPs contain two characteristic structural domains: an OmpR DNA-binding domain and a bacterial transcription activation domain (BTAD) (Tanaka et al. 2007). The SARPs include the positive regulators ActII-ORF4 (Fernández-Moreno et al. 1991) and RedD (Wilson et al. 2001) in *S. coelicolor* and DnrI (Parajuli et al. 2005) in *S. peucetius*. In addition, some CSRs encode proteins belonging to another family, called the LuxR family, which is often found in Gram-negative bacteria (Lei et al. 2007). LuxR-type regulators contain a nucleotide triphosphate (NTP) binding motif at the N-terminus and a helix-turn-helix (HTH) motif at the C-terminus; examples include the positive regulators PimR (Antón et al. 2004) in *Streptomyces natalensis* and PikD (Xue et al. 1998) in *Streptomyces venezuelae*. There are many additional families of regulatory proteins, including the LysR and TetR families. The CSRs are shown in Table 1. Huang et al. (2005) reported that some CSRs can also control the expression of pleiotropic genes, and some pleiotropic regulators can affect the expression of CSRs. In this paper, we will not describe the cross-regulation between various types of regulators.

tylR, tylP, tylQ, tylS and tylT

Eli Lilly and Company obtained the tylosin biosynthetic gene cluster from *Streptomyces fradiae*. Five regulators of this cluster are all CSRs. Sequence analysis identified TylP as a γ-butyrolactone (GBL) signal receptor (Bate et al. 1999; Wilson et al. 2001), and there were several indications that TylP is an effector-binding regulator and a regulator of tylosin biosynthesis (Bignell et al. 2007). The protein TylQ was reported to be a transcriptional repressor that blocked tylosin biosynthesis by controlling the expression of *tylR* (Stratigopoulos and Cundliffe 2002). Studies revealed that *tylT* and *tylS* encode proteins belonging to the SARPs family (Bate et al. 1999). Bate et al. (2006) identified a new regulatory gene, *tylU*, in the tylosin biosynthetic gene cluster. Targeted disruption of *tylU* decreased tylosin yield by approximately 80%, demonstrating that *tylU* is a positive regulatory gene for tylosin biosynthesis. TylR, a CSR, was able to regulate the core polyketide genes, but it primarily affected tylosin biosynthesis (Bate et al. 1999). The tylosin biosynthetic gene cluster is shown in Fig. 1.

dnrI, dnrO and dnrN

A previous report (Parajuli and Moon 2002) has demonstrated that the DNR producer *S. peucetius* possesses two DNA segments, *dnrR1* and *dnrR2*. Sequence analysis of *dnrR1* and the subsequent inactivation of *dnrI*, which is contained within *dnrR1*, suggested the involvement of *dnrI* in the transcription of the biosynthetic genes of DNR. The disruption of *dnrI* resulted in the absence of DNR production (Madduri and Hutchinson 1995), and the overexpression of *dnrI* under the control of the strong ermE* promoter increased the production of DNR (Malla et al. 2010). These results indicated that *dnrI* positively regulates DNR biosynthesis. *dnrN* encodes a response regulator (RR) of UhpA-LuxR superfamily of regulatory proteins and has a motif that is highly similar to the HTH DNA-binding motif. An earlier study showed that reintroduction of *dnrN* into a *dnrI:aphII* mutant failed to restore DNR production. This suggested that DnrN activates the transcription of *dnrI* in the regulatory cascade; *dnrI*, in turn, positively triggers the transcription of DNR biosynthetic genes (Otten et al. 1995). *dnrO*, which is a negative regulatory gene, encodes a DNA-binding protein. DnrO has been shown to regulate antibiotic yield in *S. peucetius* by positively controlling *dnrN* (Otten et al. 2000; Parajuli and Moon 2002).

rapH, rapG and rapY

DNA sequence analysis of *rapH* and *rapG* in *Streptomyces hygroscopicus* revealed that RapH and RapG share significant similarity with two positive transcriptional families, the LAL and AraC families, respectively (Kuščer et al. 2007). RapH contained a DNA-binding motif and an ATP-binding site, while RapG contained a HTH DNA-binding motif (Yoo et al. 2015). In one study, antibiotic production was increased by 50% only if copies of both *rapH* and *rapG* under the control of their native promoter regions were introduced. Further, the complementation of *rapH* and *rapG* deletion mutants under the control of their native promoters led to a restoration of rapamycin production to parental levels (Yoo et al. 2015). The overexpression of both genes led to an abundant rapamycin synthesis, while the deletion of *rapG* and *rapH* caused a total shutdown of antibiotic production, suggesting that *rapG* and *rapH* play

Table 1 Regulatory genes involved in pathway-specific regulation

Regulatory gene(s)	Gene(s) from	Product(s) of regulation	Function	Notes	References
actII-ORF4	*S. coelicolor*	ACT	+	Encodes a SARP	Fernández-Moreno et al. (1991)
mmyR	*S. coelicolor*	Methylenomycin	−	A gene adjacent to *mmyR* provides positive regulation	Arias et al. (1999)
cdaR	*S. coelicolor*	Mmy and CDA	+	Encodes a SARP	Bibb (2005)
redD and *redX*	*S. coelicolor*	RED	+	Encode SARPs, and the transcription of *redX* regulates the transcription of *redD*	Romero-Rodríguez et al. (2015), Takano et al. (1992) and Wilson et al. (2001)
gdmRI and *gdmRII*	*S. hygroscopicus* 179977	Geldanamycin	+	Regulate the transcription of *pks*, *gdmF* and *gdnA*, which are involved in biosynthesis of geldanamycin	(He et al. 2008)
tylR, *tylS*, *tylP*, *tylQ* *tylT* and *tylU*	*S. fradiae*	Tylosin	*tylR*, *tylS*, *tyl T* and *tylU*: + *tylQ*: −	*tylT* and *tylS* encode SARPs, and TylP is similar to γ-butyrolactone receptor proteins	Bate et al. (1999, 2006) and Stratigopoulos and Cundliffe (2002)
aveR, *aveR1*, *aveR2* and *aveT*	*S. avermitilis*	Avermectin	+	*aveR* contains a HTH motif; AveT belongs to the TetR family and activates transcription of *aveR*	Ikeda et al. (2003)
alpT, *alpU*, *alpV*, *alpW* and *alpZ*	*S. ambofaciens*	Alpomycin	*alpT*, *alpU*, and *alpV*: +; *alpW*: −	*alpT*, *alpU* and *alpV* encode SARPs, *alpW* encodes a transcriptional repressor protein, and *alpZ* encodes a γ-butyrolactone receptor protein	Aigle et al. (2005)
ccaR	*S. clavuligerus*	Cephamycin C and clavulanic acid	+	Encodes a SARP	Pérez-Llarena et al. (1997)
pimR	*S. natalensis*	Pimaricin	+	Encodes a LuxR family protein, does not regulate its own transcription	Antón et al. (2004)
pikD	*S. venezuelae*	Pikromycin	+	Encodes a LuxR family protein	Xue et al. (1998)
monH, *monRI* and *monRII*	*S. cinnamonensis*	Monensin	+	MonH is similar to PikD, and *monRI* encodes a SARP	Oliynyk et al. (2003)
spbR	*S. pristinaespiralis*	Pristinamycin	+	SpbR is a γ-butyrolactone receptor	Mast et al. (2015)
papR1-R5	*S. pristinaespiralis*	Pritinamycin	*papR1*, *papR2* and *papR4*: +; *papR3* and *papR5*: −	PapR1, PapR2, and PapR4 are SARPs, and PapR3 and PapR5 belong to the TetR family	Mast et al. (2015)
*jadR** and *jadR3*	*S. venezuelae*	Jadomycin B	−	JadR* is a TetR-like protein, and JadR3 represses *jadR2* and *jadR3* but activates *jadR1*	Yang et al. (2001), Zhang et al. (2013) and Zou et al. (2014)
nysRI-RIII	*S. noursei* ATCC	Nystatin	+	Deletion of *nysRI* abolishes the transcription of *nysRII-III*	Sekurova et al. (2004)
amph RI- RIII	*S. nodosus*	Amphotericin	+	All contain a HTH motif in C-terminal	Carmody et al. (2004)
fscRI-RIII	*S. pp.* FR008	Candicidin	+	Encodes a LuxR family protein	Chen et al. (2003)
dnrI, *dnrO* and *dnrN*	*S. peucetius*	Daunorubicin	*dnrI*: +; *dnrO*: −; *dnrN*: +	DnrN is a RR belonging to the Uhp-LuxR superfamily and activates the transcription of *dnrI*, and DnrO positively controls *dnrN*	Otten et al. (2000) and Parajuli and Moon (2002)
strR	*S. griseus*	Streptomycin	+	Regulates streptomycin by activating the expression of *strA* and *strB*	Distler et al. (1987)
rapH, *rapG* and *rapY*	*S. hygroscopicus*	Rapamycin	*rapH*, *rapG*: + *rapY*: −	RapG and RapY each contain a HTH motif, and RapH contains a DNA-binding motif and an ATP-binding site	Yoo et al. (2015)
srrX, *srrY*, *srrZ* and *srrB*	*S. rochei*	Lankamycin and lancadicin	*srrX* and *ssrY*: + for both *ssrZ*: + for lankamycin *srrB*: − for both	*srrY* and *srrZ* encode SARPs, and *ssrY* positively regulates *ssrZ*	Arakawa et al. (2007) and Suzuki et al. (2010)

Table 1 continued

Regulatory gene(s)	Gene(s) from	Product(s) of regulation	Function	Notes	References
scbR and *scbR2*	*S. coelicolor*	ACT, RED, CDA and yCPK	ACT, CDA and RED: + yCPK: −	ScbR is a γ-butyrolactone receptor protein, and ScbR2 is an antibiotic receptor protein	Li et al. (2015a)
polR and *polY*	*S. cacaoi*	Polyoxin	+	Both encode SARPs, and the transcription of *polR* is positively regulated by *polY*	Hwang et al. (2003)
aur1PR3 and *aur1PR4*	*S. aureofaciens*	Auricin	+	Both encode SARPs, *aur1PR3* is controlled by Aur1R, and Aur1P directly regulates the expression of *aur1PR4*	Rehakova et al. (2013)
barA, *barB* and *varR*,	*S. virginiae*	Virginiamycin	−	BarA, BarB and VarR are TetR-like regulators, and the transcription of *barB* is tightly repressed by BarA	Matsuno et al. (2003), Nakano et al. (2000) and Namwat et al. (2001)
vmsS, *vmsT* and *vmsR*	*S. virginiae*	Virginiamycin M and virginiamycin S	*vmsS* and *vmsR*: + for virginiamycin M and virginiamycin S; *vmsT*: − for virginiamycin M	VmsS and VmsR are SARPs, and VmsT is a RR of a TCS	Pulsawat et al. (2009)
aur1R	*S. aureofaciens*	Auricin	−	*aur1R* encodes a homolog of the TetR family, and Aur1R represses the expression of *aur1P*	Novakova et al. (2010)
fdmR1	*S. griseus*	Fredericamycin	+	Encodes a homologue of SARPs	Chen et al. (2008)
thnI	*S. cattleya*	Thienamycin	+	ThnI resembles LysR-type transcriptional activators and contains a HTH motif	Rodríguez et al. (2008)
thnU	*S. cattleya*	Cephamycin C	+	Encodes a SARP	Rodríguez et al. (2008)
asuR1, *asuR2*, *asuR4* and *asuR6*	*S. nodosus*	Asukamycin	+	AsuR1 and AsuR6 belong to the LuxR family, AsuR2 belongs to the TetR family, and asuR5 encodes a SARP	Xie et al. (2012)
farR3 and *farR4*	*S. lavendulae* FRI-5	Indigoidine	*farR3*: + *farR4*: −	Both encode SARPs, FarR3 positively controls the biosynthesis of indigoidine, and FarR4 negatively controls the expression of *farX*, *farA*, *farR1* and *farR2*	Kitani et al. (2008) and Kurniawan et al. (2014)
papR6	*S. pristinaespiralis*	Pritinamycin II	+	PapR6 is an orphan RR	Dun et al. (2015) and Mast et al. (2015)
redZ	*S. coelicolor*	RED	+	Encodes a NarL-type RR, and the transcription of *redD* depends on *redZ* and the translation of *redZ* depends on *bldA*	Guthrie et al. (1998) and Wang et al. (2009)
ssaA	*Streptomyces* sp. strain SS	Sansanmycin	+	SsaA has a N-terminal fork head-associated (FHA) domain and a C-terminal LuxR-type HTH motif	Li et al. (2013)
vlmI	*S. viridifaciens*	Valanimycin	+	*vlmI* encodes a SARP and can complement a *redD* mutation;	Garg and Parry (2010)
nanR1, *nanR2* and *nanR4*	*S. nanchangensis*	Nanchangmycin	*nanR1*, *nanR2:* + *nanR4:* −	*nanR1* and *nanR2* encode SARPs, and *nanR4* is an AraC-family transcriptional regulator and represses the transcription of *nanR1* and *nanR2*	Yu et al. (2012)

+ Represents positive regulation, − represents negative regulation

active roles in antibiotic biosynthesis. Furthermore, these genes cannot exert regulatory effects on the rapamycin biosynthetic gene cluster independent of each other. Yoo et al. (2015) found that the overexpression of *rapY* caused a drastic reduction in antibiotic production, while deletion of *rapY* increased antibiotic production by approximately fivefold. RapY, which contains an HTH motif near its N-terminus, plays a negative role in rapamycin production. The antibiotic regulatory genes in the biosynthetic gene cluster of rapamycin are shown in Fig. 2.

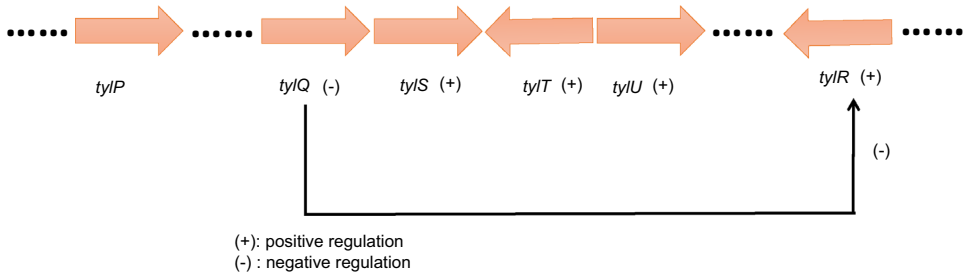

Fig. 1 Tylosin biosynthetic gene cluster of *S. fradiae*. Five regulatory genes are shown: TylP is a GBL signal receptor. TylQ is a transcriptional repressor and blocks tylosin biosynthesis by controlling the expression of *tylR*. *tylT* and *tylS* encode cluster-situated regulatory proteins of the SARPs family. *tylU* is a positive regulatory gene of tylosin biosynthesis. TylR is also a CSR

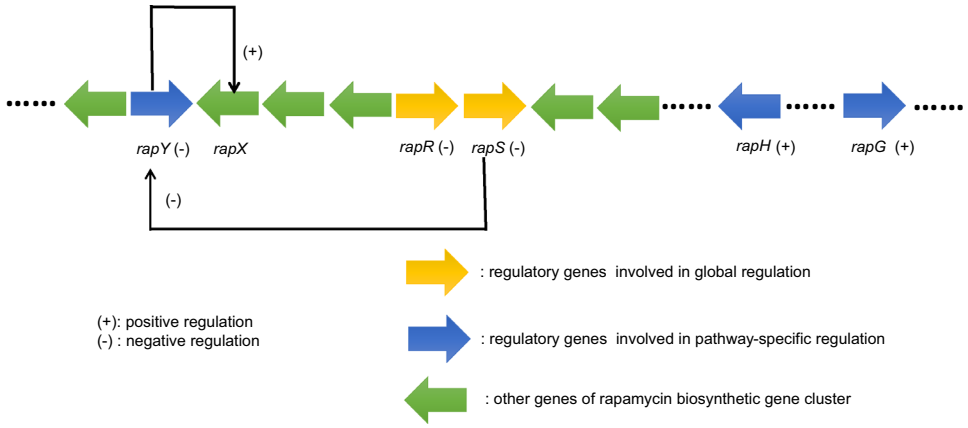

Fig. 2 The location of regulatory genes in rapamycin biosynthetic gene cluster of *S. hygroscopicus*. RapY inhibits the transcription of *rapX* which is an ABC-transporter gene. Together, *rapS* and *rapR* negatively regulate most of the rapamycin biosynthetic genes. RapS also represses the expression of *rapY*. RapH and RapG positively regulate rapamycin biosynthesis

polY and polR

Li et al. (2009) found that the deletion of *polR* completely blocked polyoxin (POL) biosynthesis, which was complemented by introducing a copy of *polR* into the mutant. Similarly, the yield of POL was found to increase with the presence of an additional copy of *polR* in the mutant. PolR is necessary for the transcription of many structural genes in the POL biosynthetic gene cluster. Another CSR, *polY* in *S. cacaoi*, positively controls the production of this antibiotic. Both *polR* and *polY* encode proteins belonging to the SARPs family, and the expression of *polR* depends on the activity of PolY (Kitani et al. 2001).

aur1R, aur1P, aur1PR3 and aur1PR4

Some studies (Novakova et al. 2010, 2011; Rehakova et al. 2013) described four genes regulating auricin production in *Streptomyces aureofaciens*. *aur1R* encodes a homologue similar to the members of the TetR family. Antibiotic production was much higher in the disrupted strain than in the parental strain, which suggested a negative regulatory effect of *aur1R* on auricin production (Novakova et al. 2010). Aur1R can specifically negatively control the expression of *aur1P*, and this repression is released by auricin or its intermediates. In another experiment (Rehakova et al. 2013), *aur1P* was deleted from the chromosome, and no auricin was produced in the mutant, which suggested that *aur1P* is critical for the biosynthesis of auricin and exerts a positive effect on the expression of the biosynthetic genes of auricin. Aur1P belongs to the OmpR subfamily, which is similar to the RRs of two-component systems (TCSs). *aur1PR3* and *aur1PR4* encode proteins that are highly similar to those belonging to SARP family. The disruption of *aur1PR3*, an activator of auricin, resulted in a dramatic decrease in antibiotic production compared to the wild-type parent. Additionally, the expression of *aur1PR3* was controlled by Aur1R (Novakova et al. 2011). Aur1P directly regulated the expression of *aur1PR4* because its promoter was dependent on Aur1P (Rehakova et al. 2013).

thnI and thnU

There are two regulators, *thnI* and *thnU*, located in the thienamycin gene cluster of *Streptomyces cattleya*. ThnI is similar to the members of LysR family, and they all have a highly conserved HTH DNA-binding domain. A deletion mutant constructed by gene replacement failed to produce thienamycin, thereby revealing the importance of *thnI* in

thienamycin synthesis. Gene expression analysis of the thienamycin gene cluster demonstrated that ThnI is a positive regulator and that it can control the expression of several genes involved in the assembly and export of thienamycin (Rodríguez et al. 2008). *thnU* encodes a positive regulator that belongs to the SARPs family. HPLC–MS analysis of a *thnU* mutant constructed using the same method that was used for *thnI* indicated that inactivation of *thnU* resulted in a loss of the production of Cephamycin C, whereas thienamycin synthesis was not affected. These results revealed the positive role of ThnI in thienamycin biosynthesis and the relevance of ThnU in cephamycin C biosynthesis (Rodríguez et al. 2008).

Regulatory genes involved in pleiotropic regulation

Many regulatory genes, which are mostly located outside of biosynthetic gene clusters, have pleiotropic (or global) effects on the production of multiple secondary metabolites or on both secondary metabolites and morphological development. In *Streptomyces*, the most abundant pleiotropic regulators belong to the TCSs, which are the predominant signal transduction systems in bacteria (Stock et al. 2000; Hakenbeck and Stock 1996). Other pleiotropic regulators can regulate antibiotic production in association with many small molecules called γ-butyrolactones (GBLs). This paper divides pleiotropic regulators into two subtypes. One type includes TCSs, orphan response regulation [e.g., *farR1* (Kitani et al. 2008)], orphan histidine kinase regulation [e.g., *ohkA* (Lu and Jiang 2013)] and other special TCSs [e.g., *abrC1/C2/C3* (Yepes et al. 2011)]. This subtype of pleiotropic regulatory genes is shown in Table 2a. The other includes the pleiotropic regulators closely associated with GBLs. To date, approximately 13 GBLs have been discovered, including A-factor from *S. griseus* (Ohnishi et al. 1999), IM-2 from *S. lavendulae* (Sato et al. 1989), SCB1, SCB2 and SCB3 (Hsiao et al. 2009; Takano et al. 2005), VBs from *S. virginiae* (Yamada et al. 1987) and factor 1 from *S. viridochromogenes* (Sato et al. 1989). Moreover, a few pleiotropic regulators contain a TPR structural domain [such as *nsdA* (Yu et al. 2006)], a protein repeat sequence consisting of 34 amino acids, which encodes an HTH secondary structural fragment. This type of pleiotropic genes is shown in Table 2b.

For TCSs, there are two types of kinase super-families in *Streptomyces* that regulate secondary metabolites. One is the histidine kinase family. This family consists of two proteins, a phosphate donor—HK (histidine kinase) and a phosphate receptor—RR. Most of the HKs that belong to membrane-spanning proteins have three functional structural domains: a sensing domain, a transmitter domain and an ATPase domain. RRs contain two domains: a receiver domain and an effector domain. A HK activates itself by first phosphorylating its conserved histidine residues and then transferring the phosphate groups to the conserved asparagine acid residues of the RR. The phosphorylated RR then regulates the expression of other genes (Lu and Jiang 2013). This signal transduction process is shown in Fig. 3. Genes belonging to this family include *afsQ1/afsQ2* (Ishizuka et al. 1992), *cutR/cutS* (Tanaka et al. 2007) and *ecrA1/ecrA2* (Huang et al. 2001). The other type is the serine/threonine and tyrosine kinase family, whose members transmit signals to regulate secondary metabolism via a series of cascade reactions, such as phosphorylation or dephosphorylation of proteins triggered by external environmental changes. AfsR/AfsS (Tanaka et al. 2007) belongs to the serine/threonine and tyrosine kinase family. The genomic sequence analysis of *S. coelicolor* which is a model strain was performed in 2002. Bioinformatic analysis revealed that *S. coelicolor* contains many TCSs, encompassing 84 HKs and 80 RRs. Of these, 67 HKs have been matched with 67 RRs and are adjacent to genes encoding RRs, while the remaining TCSs are orphan HKs and orphan RRs (Hutchings et al. 2004).

nsdA and nsdB

The pleiotropic negative regulatory gene *nsdA* can be found in many *Streptomyces* spp. The *nsdA* genes from various *Streptomyces* spp. share 77–100% similarity with each other, and *nsdA* homologous genes are also present in many *Streptomyces* strains (Yu et al. 2006). NsdA, which contains a TPR structural domain, plays a negative role in sporulation, morphological differentiation and antibiotic synthesis. The overproduction of actinorhodin (ACT), calcium-dependent antibiotic (CDA) and methylenomycin was detected in a *nsdA* mutant, and the deletion of the *nsdA* in *Streptomyces lividans* also resulted in the expression of silent ACT biosynthetic genes. These results indicated that NsdA may silence the ACT biosynthetic gene cluster by repressing the expression of CSRs (Yu et al. 2006). There is also a TPR-like structural domain in the *nsdB* gene product whose disruption gives rise to ACT production, but NsdB does not affect morphological differentiation. The deletion of *nsdB*, which is a negative regulator of CDA, resulted in an increase in CDA production. *nsdB* has been shown to control antibiotic biosynthesis along with *nsdA* but has no influence on the expression of *nsdA* at the RNA level (Yu et al. 2006).

afsR2 and afsB

The protein encoded by *afsR2* is 63 amino acids long (Lee et al. 2000), and AfsR2 is a positive regulator of ACT and undecylprodigiosin (RED). Lee et al. (2000) cloned a regulatory gene from *S. avermitilis* that was homologous to

Table 2 Regulatory genes involved in pleiotropic regulation

Regulatory gene(s)	Gene(s) from	Product(s) of regulation	Function	Notes	References
a)					
afsQ1/afsQ2	*S. lividans*	ACT	+		Ishizuka et al. (1992)
afsK/afsR	*S. coelicolor* and *S. griseus*	ACT	+	AfsR is the transcriptional activator of *afsS*, which can activate *actII-ORF4*	Atsushi et al. (1994)
afsR-p/afsS	*S. peucetius*	Adriamycin	+		Parajuli et al. (2005)
cutR/cutS	*S. coelicolor* and *S. lividans*	ACT	−		Chang et al. (1996)
ecrA1/ecrA2 and *ecrE2/ecrE1*	*S. coelicolor*	RED	+	Coordinates with the expression of *redD*	Huang et al. (2001)
orfX/orf41	*S. avermitilis*	Avermectin	+	*orfX* exerts regulation by itself or by the collaboration of *orf41* with *orfX*	Hwang et al. (2003)
phoR/phoP	*S. coelicolor* and *S. lividans*	ACT and RED	−	PhoP belongs to the Ormp family	Sola-Landa et al. (2003)
valP/valQ	*S. hygroscopicus* 5008	Validamycin			Bai et al. (2006)
absA1/absA2	*S. coelicolor*	CDA, yCPK and albaflavenone	−		Sheeler et al. (2005)
rapR/rapS	*S. hygroscopicus*	Rapamycin	−	RapS represses the expression of *rapY*	Yoo et al. (2015)
rapA1/rapA2	*S. coelicolor*	ACT and yCPK	+	The regulation of RapA1/A2 depends on ActII-ORF4 and KasO	Lu et al. (2007)
draK/R	*S. coelicolor*	ACT, yCPK and RED	ACT: + RED and yCPK: −	DraR binds to the promoter regions of *actII-ORF4* and *cpkO*	Rodríguez et al. (2013)
abrA1/A2	*S. coelicolor*	ACT, CDA and RED	−		Yepes et al. (2011)
SCO0203/0204	*S. coelicolor*	ACT	−		Wang et al. (2009b)
ohkA	*S. coelicolor*	ACT, CDA and RED	−	No identified RR matches OhkA	Lu et al. (2011)
aur1P	*S. aureofaciens*	Auricin	+	*aur1P* encodes a protein similar to the RRs	Novakova et al. (2005)
farR1	*S. lavendulae* FRI-5	Nucleoside antibiotics and indigoidine		FarR1 is an orphan RR	Kitani et al. (2008)
glnR	*S. coelicolor*	RED and ACT	+	GlnR belongs to the OmpR family and indirectly regulates the production of antibiotics in response to changes in nitrogen availability	Pullan et al. (2011)
SCO3818	*S. coelicolor*	ACT	−	*SCO0203* can phosphosphorylate *SCO0204* and *SCO3818*, and there is a functional correlation between *SCO0203* and *SCO3818*	Wang et al. (2009b)
jadR1/jadR2	*S. venezuelae*	Jadomycin B	*jadR1*: + *jadR2*: −	The *jadR1* and *jadR2* genes represent a novel TCS linking antibiotic synthesis to stress; *jadR1* encodes a RR; *jadR2* encodes a TetR-like protein, and JadR2 is a pseudo γ-butyrolactone receptor	Yang et al. (2001)
abrC1/C2/C3	*S. coelicolor*	ACT, CDA and RED	+	AbrC1 and AbrC2 are HKs, and AbrC3 is a RR	Yepes et al. (2011)

Table 2 continued

Regulatory gene(s)	Gene(s) from	Product(s) of regulation	Function	Notes	References
b)					
nsdA and *nsdB*	*S. coelicolor*	ACT and CDA	−	Each encodes a protein containing a TPR structure	Yu et al. (2006)
tcrA	*S. coelicolor*	All secondary metabolites	−		Liu and Yang (2006)
afsR2	*S. lividans* and *S. coelicolor*	ACT and RED	+		Lee et al. (2000)
afsB	*S. lividans* and *S. coelicolor*	ACT, methylenomycin, CDA and RED	+		Horinouchi et al. (1989)
barX	*S. virginiae*	Virginiamycin		BarX is an AfsA-like protein	Bate et al. (1999) and Pulsawat et al. (2007)
farA	*S. lavendulae*	Nucleoside antibiotics	−	IM-2 binds to the FarA receptor to regulate the signal transduction of secondary metabolism	Kitani et al. (2001)
arpA	*S. griseus*	Streptomycin	−	ArpA is an A-factor receptor protein	Hong et al. (2007) and Kato et al. (2004)
adpA	*S. griseus*	Streptomycin	+	Encodes an Arac/XylS family protein and has two HTH motifs at the C-terminal	Higo et al. (2012), Ohnishi et al. (1999, 2005) and Zhu et al. (2005)
bldD	*S. coelicolor*	ACT, indigoidine, CDA and methylenomycin	+	BldD has a C-terminal domain of unknown function and an N-terminal domain that mediates DNA binding and dimerization	Den Hengst et al. (2010)
bldA	*S. coelicolor*	ACT	+	BldA regulates the production of antibiotics by controlling the activator ActII-ORF4	Fernández-Moreno et al. (1991)
crp	*S. coelicolor*	ACT, RED and CDA	+	Crp is a member of the cAMP receptor protein/fumarate-nitrate-reductase family of regulators	Gao et al. (2012)
wblA	*S. coelicolor*	ACT, RED and CDA	−	WblA is a protein of the WhiB family	Kang et al. (2007)
atrA	*S. coelicolor*	ACT	+	AtrA is a TetR-like protein, AtrA positively controls the transcription of *actII-ORF4*	Li et al. (2015b)
rrdA	*S. coelicolor*	RED and ACT	RED: − ACT: +	RrdA belongs to the TetR family, and RrdA negatively regulates RED by controlling the abundance of RedD mRNA	Ou et al. (2009)
avaR3	*S. avermitilis*	Avermectin and filipin	Avermectin: +; filipin: −	AvaR3 is a γ-butyrolactone autoregulator receptor homologue	Miyamoto et al. (2011)
cprA	*S. coelicolor*	ACT and RED	+	Encodes an AprA analogue	Onaka et al. (1998)
cprB	*S. coelicolor*	ACT	−	CprB shows high sequence similarity to CprA	Onaka et al. (1998)

+ Represents positive regulation, − represents negative regulation

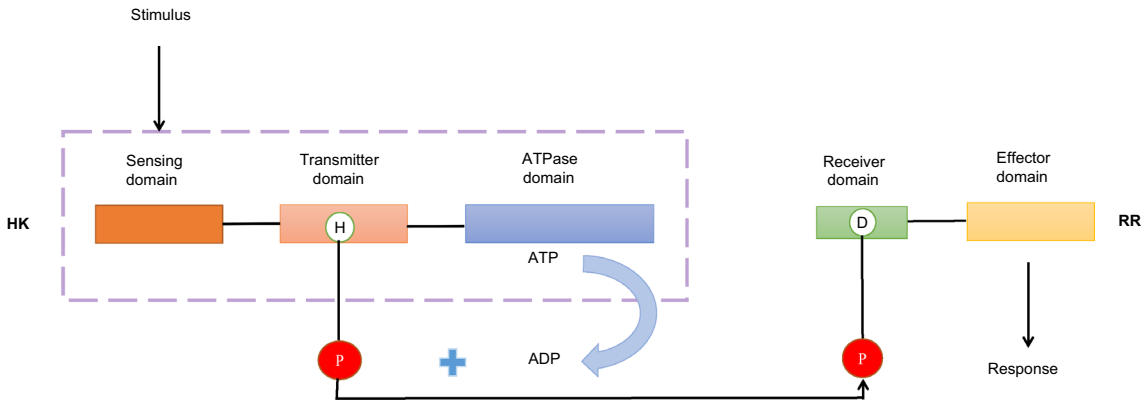

Fig. 3 The process of signal transduction in TCSs

afsR2 from *S. lividans* and *S. coelicolor*. The integration of multiple copies of *afsR2* into the wild-type strain resulted in approximately twofold overproduction of avermectin. ACT and RED can be regulated by pleiotropic regulatory genes in addition to synthetic gene clusters. *afsB* encodes a DNA-binding protein and is a pleiotropic regulator that is essential for ACT biosynthesis. An increase in copies of *afsB* significantly improved the production of ACT and RED. The introduction of *afsB* into *S. lividans* triggered the transcription of ACT biosynthetic genes that were otherwise silent (Horinouchi et al. 1989). *afsB* provides positive regulation by stimulating its target genes and can trigger RED biosynthesis in *S. lividans*.

cprA and cprB

In *S. coelicolor*, the gene products of *cprA* and *cprB* are highly similar to each other. Onaka et al. (1998) found that the deletion of *cprA* led to a sharp reduction of ACT and RED. The introduction of *cprA* into the parental strain resulted in increased antibiotic production. Those results demonstrated that *cprA* positively regulated the biosynthesis of ACT and RED (Onaka et al. 1998). The disruption of *cprB* led to a precocious overproduction of ACT, but no change was detected in the production of RED. These results revealed that *cprB* only negatively regulated the biosynthesis of ACT (Onaka et al. 1998). Another study showed that *cprA* and *cprB* activated antibiotic biosynthesis in *S. coelicolor* via the GBL quorum-sensing pathway (Bhukya et al. 2014).

adpA and arpA

adpA, which is a pleiotropic regulator, encodes a 405-amino-acid protein containing a HTH DNA-binding motif in its central region. AdpA showed high sequence similarity to the transcriptional regulators of the AraC/XylS family (Gallegos et al. 1997). To determine the function of *adpA* in *S. griseus*, a disruption strain was generated using in-frame deletion. No streptomycin was detected in the disrupted strain, but streptomycin production was recovered when *adpA* was introduced, showing that *adpA* exerted a positive effect on streptomycin biosynthesis (Higo et al. 2012; Ohnishi et al. 1999). ArpA contains a region resembling the HTH motif that is present in many transcriptional families. ArpA is an A-factor receptor protein that negatively controls the production of streptomycin in *S. griseus*. ArpA behaved as a repressor-type regulator for streptomycin production (Onaka et al. 1995). *adpA*, *arpA* and A-factor form a widespread regulatory cascade in *Streptomyces*, termed the A-factor-*adpA*-*arpA* regulatory cascade. There is a model for the A-factor regulatory cascade that leads to streptomycin biosynthesis (Ohnishi et al. 1999): A-factor gradually accumulates, and when the concentration of A-factor reaches a certain point, it binds ArpA and causes ArpA to dissociate from the promoter, thus leading to the transcription and translation of *adpA*. AdpA then activates the transcription of *strR*. Therefore, the induction of StrR positively regulates the transcription of most of the streptomycin biosynthetic genes (Retzlaff and Distler 1995).

afsQ1/afsQ2 and cutR/cutS

afsQ1/afsQ2 is representative of the TCSs, with *afsQ1* encoding an aspartic acid RR and AfsQ2 belonging to the sensing kinases. AfsQ2, located in the membrane, is autophosphorylated at His-294 upon sensing an environmental signal, and the signal is then transferred to the Asp-52 residue of the AfsQl protein in the cytoplasm (Ishizuka et al. 1992). *afsQ1/afsQ2* plays a pleiotropic role in the secondary metabolism of antibiotics. *cutR/cutS* is the second TCS found in *Streptomyces* that represses secondary metabolism, and *cutR/cutS* also negatively regulates antibiotic production. The time taken to produce ACT is 20 h shorter in the *cutR/cutS* mutant than in the parental

strain. The inactivation of *cutR/cutS* triggers an increase in ACT production, and the introduction of *cutR* reverses this change (Chang et al. 1996).

afsR-p/afsS

Parajuli et al. (2005) isolated the *afsR-p* gene from *S. peucetius* ATCC 27952, and it had greater than 50% sequence similarity to *afsR* from *S. coelicolor*. The overproduction of doxorubicin, γ-actinorhodin, clavulanic acid and streptomycin (slight), respectively, was detected in strains of *S. peucetius*, *S. lividans*, *S. clavuligerus* and *S. griseus* strains that carried the *afsR-p* gene (Parajuli et al. 2005). This demonstrated that AfsR-p may activate various CSRs in antibiotic biosynthetic gene clusters in different ways, leading to the speculation that phosphorylated AfsR-p binds to the promoter region of *afsS* and that the latter then triggers other regulators to induce the production of certain secondary metabolites (Parajuli et al. 2005).

orfX/orf41 and phoR/phoP

Disruption of the *orfX* gene resulted in a significant but incomplete loss of the production of avermectin in *S. avermitilis* (Hwang et al. 2003). The increase in avermectin may result from the role of *orfX* itself or from the collaboration of *orf41* with *orfX*. Recently, a new TCS, *phoR-phoP*, was found in *S. lividans* and *S. coelicolor*, and PhoP was indeed a member of the OmpR family. The PhoR-PhoP system may activate the formation of ACT and RED via a specific repressor protein with phosphate-controlled promoters, acting via a cascade mechanism (Sola-Landa et al. 2003).

valP/valQ and rapR/rapS

valP and *valQ*, which can regulate validamycin in *S. hygroscopicus* 5008, may encode a TCS regulatory protein consisting of a HK and a Sigma B PP2C-like phosphatase (Bai et al. 2006). RapR and RapS in *Streptomyces rapamycinicus* ATCC 29253 share high sequence identities with the RRs and HKs, respectively, of TCSs. Gene expression analysis demonstrated that most of the rapamycin biosynthetic genes were negatively controlled by *rapS* (probably in collaboration with *rapR*) and *rapY* (a CSR for the biosynthesis of rapamycin) (Yoo et al. 2015). In addition, RapS represses the expression of *rapY* gene and RapR/S is a repressor of rapamycin biosynthesis.

rapA1/A2, abrA1/A2 and abrC1/C2/C3

The *rapA1* in *S. coelicolor* encodes a protein that belongs to the OmpR family, while the sequence of RapA2 shows characteristics that are typical of HKs (Lu et al. 2007). Lu et al. (2007) found that RapA1/A2 is an activator of ACT and a yellow cryptic polyketide (yCPK). The effect exerted by *rapA1/A2* on the biosynthesis of these antibiotics may also depend on two CSRs, *actII-ORF4* and *kasO*. The disruption of *rapA1/A2* and subsequent experiments with the disrupted strain indicated that *rapA1/A2* was a positive TCS regulator of ACT [which is encoded by a type II polyketide synthase (PKS)] and yCPK [which is encoded by a type I PKS in *S. coelicolor* (Lu et al. 2007)]. *abrA1/A2* is also a TCS regulator that negatively regulates ACT, RED and CDA in *S. coelicolor*. However, *abrC1/C2/C3* is composed of two HKs and one RR that positively regulate the abovementioned antibiotics. AbrC1 and AbrC2 are HKs, while AbrC3 is a RR (Yepes et al. 2011).

ohkA

OhkA was reported to be an orphan HK in *S. coelicolor* that repressed the biosynthesis of five known secondary metabolites: ACT, RED, CDA, yCPK and albaflavenone (Lu and Jiang 2013). The deletion of *ohkA* in *S. coelicolor* caused a drastic increase in the biosynthesis of antibiotics, especially of ACT and CDA (Räty et al. 2002). OhkA negatively regulates secondary metabolites by repressing CSRs in *S. coelicolor*.

Other regulatory genes

In addition to CSRs and pleiotropic regulators, there are many other regulatory genes that deserve attention. These include *barZ* in *Streptomyces virginiae*, which regulates virginiamycin (Pulsawat et al. 2007); *ppk* from *S. lividans* (Ghorbel et al. 2006); *hyg1* and *hyg3* (Palaniappan et al. 2006) from *S. hygroscopicus* NRRL 2388; and some regulatory genes whose functions remain unknown, such as *claR*, which is related to cephamycin C and clavulanic acid. Regulatory genes of this type are shown in Table 3.

Discussion

The regulation of secondary metabolite biosynthetic gene clusters in *Streptomyces* spp. is currently receiving substantial attention. Many transcription units are apparently regulated by several metabolite regulatory genes via transcriptional activation or repression, and biosynthetic gene clusters often collaborate with transcriptional regulators. Furthermore, it is reported that many *Streptomyces* strains can produce antibiotics. Analyses of the genomic sequences of many *Streptomyces* spp., such as *S. coelicolor* and *S. avermitilis*, have also been completed. These analyses provide a basis for post-genomic projects involving

Table 3 Other regulatory genes

Regulatory gene(s)	Gene(s) from	Product(s) of regulation	Function	Notes	References
hyg1 and *hyg3*	*S. hygroscopicus*	Hygromycin A		Hyg1 and Hyg3 show sequence similarities to AfsR and StrR, respectively	Palaniappan et al. (2006)
ppk	*S. lincolnensis*	ACT	−	Positively controlled by *phoR/phoP*	Ghorbel et al. (2006)
barZ	*S. virginiae*	Virginiamycin			Bate et al. (1999 and Pulsawat et al. (2007)
farR2	*S. lavendulae* FRI-5	Nucleoside antibiotics and indigoidine		FarR2 is a GBL receptor	Kitani et al. (2008)
claR	*S. clavuligerus*	Cephamycin C and clavulanic acid	Cephamycin C: − Clavulanic acid: +	ClaR contains two HTH motifs and is homologous with the LysR family	Pérez-Redondo et al. (1998)
stgR	*S. coelicolor*	ACT and RED	−	StgR belongs to the LysR family of transcriptional regulators and regulates antibiotics by indirectly repressing *redD* and *actII-ORF4*	Mao et al. (2013)
lndYR	*S. globisporus*	Landomycin	+	Encodes a GntR-like regulator of the YtrA subfamily	Ostash et al. (2011)

+ Represents positive regulation, − represents negative regulation

Streptomyces and will effectively promote studies of the structure, function and expression regulation of biosynthetic genes. In addition, many regulatory genes can also be expressed in other strains and can influence antibiotic production by heterologous expression combined with other methods. For example, the integration of *aveR*, *orfX*, or *afsR* in the chromosomes of *S. hygroscopicus* promoted rapamycin production by approximately 3.8-fold, 1.2-fold or slightly, respectively (Huang et al. 2011). Currently, we can effectively influence antibiotic production by manipulating regulatory genes involved in pathway-specific regulation or pleiotropic regulation in *Streptomyces*. For example, as introduced in this paper, the *aveR* mutants in *S. avermitilis* lost the ability to synthesize avermectin, whereas the overexpression of this gene increased the yield (Ikeda et al. 2003). The overproduction of ACT, CDA and methylenomycin was detected in the *nsdA* mutant of *S. coelicolor* (Yu et al. 2006). Hence, studying the regulatory mechanisms of secondary metabolite biosynthesis, inactivating the transcriptional repressors and overexpressing the transcriptional activators in natural producing strains may allow the optimization of antibiotic production. Further, it will provide a crucial theoretical basis for improving antibiotic production and using regulators to activate silent gene clusters, thereby leading to the discovery of new drugs.

Acknowledgements This work is supported by the Open Research Program of Key Laboratory of Synthetic Biology, the Chinese Academy of Sciences (SYN201612) and the Key Program of Sichuan Science and Technology Project (2017GZ0430). The authors thank all the supporting institutions.

Compliance with ethical standards

Funding This study was funded by the Open Research Program of Key Laboratory of Synthetic Biology, the Chinese Academy of Sciences (SYN201612) and the Key Program of Sichuan Science and Technology Project (2017GZ0430).

Conflict of interest All of the authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aigle B, Pang X, Decaris B, Leblond P (2005) Involvement of AlpV, a new member of the *Streptomyces* antibiotic regulatory protein family, in regulation of the duplicated type II polyketide synthase *alp* gene cluster in *Streptomyces ambofaciens*. J Bacteriol 187:2491–2500. doi:10.1128/jb.187.7.2491-2500.2005

Antón N, Mendes MV, Martín JF, Aparicio JF (2004) Identification of PimR as a positive regulator of pimaricin biosynthesis in *Streptomyces natalensis*. J Bacteriol 186:2567–2575. doi:10.1128/jb.186.9.2567-2575.2004

Arakawa K, Mochizuki S, Yamada K, Noma T, Kinashi H (2007) γ-Butyrolactone autoregulator-receptor system involved in lankacidin and lankamycin production and morphological differentiation in *Streptomyces rochei*. Microbiology 153:1817–1827. doi:10.1099/mic.0.2006/002170-0

Arias P, Fernández-Moreno MA, Malpartida F (1999) Characterization of the pathway-specific positive transcriptional regulator for actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2) as a DNA-binding protein. J Bacteriol 181:6958–6968

Atsushi M, Soon-Kwang H, Hiroshi I, Sueharu H, Teruhiko B (1994) Phosphorylation of the AfsR protein involved in secondary metabolism in *Streptomyces* species by a eukary otic-type protein kinase. Gene 146:47–56. doi:10.1016/0378-1119(94)90832-X

Bai L et al (2006) Functional analysis of the validamycin biosynthetic gene cluster and engineered production of validoxylamine A. Chem Biol 13:387–397. doi:10.1016/j.chembiol.2006.02.002

Bate N, Butler AR, Gandecha AR, Cundliffe E (1999) Multiple regulatory genes in the tylosin biosynthetic c luster of *Streptomyces fradiae*. Chem Biol 6:617–624. doi:10.1016/S1074-5521(99)80113-6

Bate N, Bignell DRD, Cundliffe E (2006) Regulation of tylosin biosynthesis involving 'SARP-helper' activity. Mol Microbiol 62:148–156. doi:10.1111/j.1365-2958.2006.05338.x

Bhukya H, Bhujbalrao R, Bitra A, Anand R (2014) Structural and functional basis of transcriptional regulation by TetR family protein CprB from *S. coelicolor A3(2)*. Nucleic Acids Res. doi:10.1093/nar/gku587

Bibb M (1996) The regulation of antibiotic production in *Streptomyces coelicolor A3(2)*. Microbiology 142:1335–1344

Bibb MJ (2005) Regulation of secondary metabolism in *streptomycetes*. Curr Opin Microbiol 8:208–215. doi:10.1016/j.mib.2005.02.016

Bignell DRD, Bate N, Cundliffe E (2007) Regulation of tylosin production: role of a TylP-interactive ligand. Mol Microbiol 63:838–847. doi:10.1111/j.1365-2958.2006.05541.x

Carmody M et al (2004) Analysis and manipulation of amphotericin biosynthetic genes by means of modified phage KC515 transduction techniques. Gene 343:107–115. doi:10.1016/j.gene.2004.08.006

Chang H, Chen MY, Shieh YT, Bibb MJ, Chen CW (1996) The *cutRS* signal transduction system of *Streptomyces lividans* represses the biosynthesis of the polyketide antibiotic actinorhodin. Mol Microbiol 21:1075–1085

Chen S et al (2003) Organizational and mutational analysis of a complete FR-008/Candicidin gene cluster encoding a structurally related polyene complex. Chem Biol 10:1065–1076. doi:10.1016/j.chembiol.2003.10.007

Chen Y, Wendt-Pienkowski E, Shen B (2008) Identification and utility of FdmR1 as a *Streptomyces* antibiotic regulatory protein activator for fredericamycin production in *Streptomyces griseus* ATCC 49344 and heterologous hosts. J Bacteriol 190:5587–5596. doi:10.1128/jb.00592-08

Den Hengst CD, Tran NT, Bibb MJ, Chandra G, Leskiw BK, Buttner MJ (2010) Genes essential for morphological development and antibiotic production in *Streptomyces coelicolor* are targets of BldD during vegetative growth. Mol Microbiol 78:361–379. doi:10.1111/j.1365-2958.2010.07338.x

Distler J, Ebert A, Mansouri K, Pissowotzki K, Stockmann M, Piepersberg W (1987) Gene cluster for streptomycin biosynthesis in *Streptomyces griseus*: nucleotide sequence of three genes and analysis of transcriptional activity. Nucleic Acids Res 15:8041–8056

Dun J et al (2015) PapR6, a putative atypical response regulator. Functions as a pathway-specific activator of pristinamycin II biosynthesis in *Streptomyces pristinaespiralis*. J Bacteriol 197:441–450. doi:10.1128/jb.02312-14

Fernández-Moreno MA, Caballero J, Hopwood DA, Malpartida F (1991) The act cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *streptomyces*. Cell 66:769–780. doi:10.1016/0092-8674(91)90120-N

Gallegos MT, Schleif R, Bairoch A, Hofmann K, Ramos JL (1997) Arac/XylS family of transcriptional regulators. Microbiol Mol Biol Rev 61:393–410

Gao C, Hindra, Mulder D, Yin C, Elliot MA (2012) Crp is a global regulator of antibiotic production in *Streptomyces*. MBio 3:1–12. doi:10.1128/mBio.00407-12

Garg RP, Parry RJ (2010) Regulation of valanimycin biosynthesis in *Streptomyces viridifaciens*: characterization of VlmI as a *Streptomyces* antibiotic regulatory protein (SARP). Microbiology 156:472–483. doi:10.1099/mic.0.033167-0

Ghorbel S, Smirnov A, Chouayekh H, Sperandio B, Esnault C, Kormanec J, Virolle M-J (2006) Regulation of *ppk* expression and in vivo function of Ppk in *Streptomyces lividans* TK24. J Bacteriol 188:6269–6276. doi:10.1128/jb.00202-06

Guthrie EP, Flaxman CS, White J, Hodgson DA, Bibb MJ, Chater KF (1998) A response-regulator-like activator of antibiotic synthesis from *Streptomyces coelicolor* A3(2) with an amino-terminal domain that lacks a phosphorylation pocket. Microbiology 144 (3):727–738. doi:10.1099/00221287-144-3-727

Hakenbeck R, Stock JB (1996) Analysis of two-component signal transduction systems involved in transcriptional regulation. Methods Enzymol 273:281–300. doi:10.1016/S0076-6879(96)73026-4

He W, Lei J, Liu Y, Wang Y (2008) Regulatory genes of geldanamycin biosynthesis. Chin J Biotechnol 24:717–722

Higo A, Hara H, Horinouchi S, Ohnishi Y (2012) Genome-wide distribution of AdpA, a global regulator for secondary metabolism and morphological differentiation in *Streptomyces*. Revealed the extent and complexity of the AdpA regulatory network. DNA Res 19:259–274. doi:10.1093/dnares/dss010

Hong B, Phornphisutthimas S, Tilley E, Baumberg S, McDowall KJ (2007) Streptomycin production by *Streptomyces griseus* can be modulated by a mechanism not associated with change in the *adpA* component of the A-factor cascade. Biotechnol Lett 29:57–64. doi:10.1007/s10529-006-9216-2

Horinouchi S, Malpartida F, Hopwood D, Beppu T (1989) *afsB* stimulates transcription of the actinorhodin biosynthetic pathway in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans*. Mol Gen Genet 215:355–357. doi:10.1007/bf00339742

Hsiao N-H et al (2009) Analysis of two additional signaling molecules in *Streptomyces coelicolor* and the development of a butyrolactone-specific reporter system. Chem Biol 16:951–960. doi:10.1016/j.chembiol.2009.08.010

Huang J, Lih CJ, Pan KH, Cohen SN (2001) Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthetic pathways in *Streptomyces coelicolor* using DNA microarrays. Genes Dev 15:3183–3192. doi:10.1101/gad.943401

Huang J et al (2005) Cross-regulation among disparate antibiotic biosynthetic pathways of *Streptomyces coelicolor*. Mol Microbiol 58:1276–1287. doi:10.1111/j.1365-2958.2005.04879.x

Huang M, Li M, Feng Z, Liu Y, Chu Y, Tian Y (2011) Enhanced rapamycin production in *Streptomyces hygroscopicus* by integrative expression of *aveR*, a LAL family transcriptional regulator. World J Microbiol Biotechnol 27:2103–2109. doi:10.1007/s11274-011-0673-y

Hutchings MI, Hoskisson PA, Chandra G, Buttner MJ (2004) Sensing and responding to diverse extracellular signals? Analysis of the sensor kinases and response regulators of *Streptomyces*

coelicolor A3(2). Microbiology 150:2795–2806. doi:10.1099/mic.0.27181-0

Hwang YS, Kim ES, Biró S, Choi CY (2003) Cloning and analysis of a DNA fragment stimulating avermectin production in various *Streptomyces avermitilis* strains. Appl Environ Microbiol 69:1263–1269. doi:10.1128/aem.69.2.1263-1269.2003

Ikeda H et al. (2003) Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat Biotech 21:526–531. http://www.nature.com/nbt/journal/v21/n5/suppinfo/nbt820_S1.html

Ishizuka H, Horinouchi S, Kieser HM, Hopwood DA, Beppu T (1992) A putative two-component regulatory system involved in secondary metabolism in *Streptomyces* spp. J Bacteriol 174:7585–7594

Kang S-H, Huang J, Lee HN, Hur YA, Cohen SN, Kim KS (2007) Interspecies DNA microarray analysis identifies WblA as a pleiotropic down-regulator of antibiotic biosynthesis in *Streptomyces*. J Bacteriol 189:4315–4319. doi:10.1128/jb.01789-06

Kato J-Y, Miyahisa I, Mashiko M, Ohnishi Y, Horinouchi S (2004) A single target is sufficient to account for the biological effects of the a-factor receptor protein of *Streptomyces griseus*. J Bacteriol 186:2206–2211. doi:10.1128/jb.186.7.2206-2211.2004

Kitani S, Yamada Y, Nihira T (2001) Gene replacement analysis of the butyrolactone autoregulator receptor (FarA) reveals that FarA acts as a novel regulator in secondary metabolism of *Streptomyces lavendulae* FRI-5. J Bacteriol 183:4357–4363. doi:10.1128/jb.183.14.4357-4363.2001

Kitani S, Iida A, Izumi T, Maeda A, Yamada Y, Nihira T (2008) Identification of genes involved in the butyrolactone autoregulator cascade that modulates secondary metabolism in *Streptomyces lavendulae* FRI-5. Gene 425:9–16. doi:10.1016/j.gene.2008.07.043

Kurniawan Y, Kitani S, Maeda A, Nihira T (2014) Differential contributions of two SARP family regulatory genes to indigoidine biosynthesis in *Streptomyces lavendulae* FRI-5. Appl Microbiol Biotechnol 98:9713–9721. doi:10.1007/s00253-014-5988-9

Kuščer E, Coates N, Challis I, Gregory M, Wilkinson B, Sheridan R, Petković H (2007) Roles of *rapH* and *rapG* in positive regulation of rapamycin biosynthesis in *Streptomyces hygroscopicus*. J Bacteriol 189:4756–4763. doi:10.1128/jb.00129-07

Lee JY, Hwang YS, Kim SS, Kim ES, Choi CY (2000) Effect of a global regulatory gene, *afsR2*, from *Streptomyces lividans* on avermectin production in *Streptomyces avermitilis*. J Biosci Bioeng 89:606–608. doi:10.1016/S1389-1723(00)80065-1

Lei J, He W, Wang Y (2007) Regulatory mechanism of morphological differentiation and secondary metabolism in *Streptomyces*. Pharm Biotechnol 14:225–229

Li R et al (2009) *polR*, a pathway-specific transcriptional regulatory gene, positively controls polyoxin biosynthesis in *Streptomyces cacaoi* subsp. asoensis. Microbiology 155:1819–1831. doi:10.1099/mic.0.028639-0

Li Q, Wang L, Xie Y, Wang S, Chen R, Hong B (2013) SsaA, a member of a novel class of transcriptional regulators, controls sansanmycin production in *Streptomyces* sp. Strain SS through a feedback mechanism. J Bacteriol 195:2232–2243. doi:10.1128/jb.00054-13

Li X, Wang J, Li SS, Ji JJ, Wang WS, Yang KQ (2015a) ScbR- and ScbR2-mediated signal transduction networks coordinate complex physiological responses in *Streptomyces coelicolor*. Sci Rep 5:14831. doi:10.1038/srep14831

Li X et al (2015b) Binding of a biosynthetic intermediate to AtrA modulates the production of lidamycin by *Streptomyces globisporus*. Mol Microbiol 96:1257–1271. doi:10.1111/mmi.13004

Liu J, Yang K (2006) Functional analyses of TcrA-a TPR-containing regulatory protein in *Streptomyces coelicolor* A3(2). Wei Sheng Wu Xve Bao 46:33–37

Lu Y, Jiang W (2013) Perspectives on two-component systems involved in regulation of secondary metabolism in *Streptomyces*. Microbiol China 40:1847–1859

Lu Y et al (2007) Characterization of a novel two-component regulatory system involved in the regulation of both actinorhodin and a type I polyketide in *Streptomyces coelicolor*. Appl microbiol biotechnol 77:625–635. doi:10.1007/s00253-007-1184-5

Lu Y et al (2011) An orphan histidine kinase, OhkA, regulates both secondary metabolism and morphological differentiation in Streptomyces coelicolor. J Bacteriol 193:3020–3032. doi:10.1128/jb.00017-11

Madduri K, Hutchinson CR (1995) Functional characterization and transcriptional analysis of the *dnrR1* locus, which controls daunorubicin biosynthesis in *Streptomyces peucetius*. J Bacteriol 177:1208–1215. doi:10.1128/jb.177.5.1208-1215.1995

Malla S, Niraula NP, Liou K, Sohng JK (2010) Improvement in doxorubicin productivity by overexpression of regulatory genes in *Streptomyces peucetius*. Res Microbiol 161:109–117. doi:10.1016/j.resmic.2009.12.003

Mao X, Sun Z, Liang B, Wang Z, Feng W, Huang F, Li Y (2013) Positive feedback regulation of *stgR* expression for secondary metabolism in *Streptomyces coelicolor*. J Bacteriol 195:2072–2078. doi:10.1128/jb.00040-13

Martín J-F, Liras P (2010) Engineering of regulatory cascades and networks controlling antibiotic biosynthesis in *Streptomyces*. Curr Opin Microbiol 13:263–273. doi:10.1016/j.mib.2010.02.008

Mast Y, Guezguez J, Handel F, Schinko E (2015) A complex signaling cascade governs pristinamycin biosynthesis in *Streptomyces pristinaespiralis*. Appl Env Microbiol 81:6621–6636. doi:10.1128/aem.00728-15

Matsuno K, Yamada Y, Lee CK, Nihira T (2003) Identification by gene deletion analysis of *barB* as a negative regulator controlling an early process of virginiamycin biosynthesis in *Streptomyces virginiae*. Arch Microbiol 181:52–59. doi:10.1007/s00203-003-0625-5

Miyamoto KT, Kitani S, Komatsu M, Ikeda H, Nihira T (2011) The autoregulator receptor homologue AvaR3 plays a regulatory role in antibiotic production, mycelial aggregation and colony development of *Streptomyces avermitilis*. Microbiology 157:2266–2275. doi:10.1099/mic.0.048371-0

Nakano H, Lee C-K, Nihira T, Yamada Y (2000) A null mutant of the *Streptomyces virginiae barA* gene encoding a butyrolactone autoregulator receptor and its phenotypic and transcriptional analysis. J Biosci Bioeng 90:204–207. doi:10.1016/S1389-1723(00)80111-5

Namwat W, Lee CK, Kinoshita H, Yamada Y, Nihira T (2001) Identification of the *varR* gene as a transcriptional regulator of virginiamycin S resistance in *Streptomyces virginiae*. J Bacteriol 183:2025–2031. doi:10.1128/jb.183.6.2025-2031.2001

Novakova R, Homerova D, Feckova L, Kormanec J (2005) Characterization of a regulatory gene essential for the production of the angucycline-like polyketide antibiotic auricin in *Streptomyces aureofaciens* CCM 3239. Microbiology 151:2693–2706. doi:10.1099/mic.0.28019-0

Novakova R, Kutas P, Feckova L, Kormanec J (2010) The role of the TetR-family transcriptional regulator Aur1R in negative regulation of the auricin gene cluster in *Streptomyces aureofaciens* CCM 3239. Microbiology 156:2374–2383. doi:10.1099/mic.0.037895-0

Novakova R, Rehakova A, Kutas P, Feckova L, Kormanec J (2011) The role of two SARP family transcriptional regulators in regulation of the auricin gene cluster in *Streptomyces aureofaciens* CCM 3239. Microbiology 157:1629–1639. doi:10.1099/mic.0.047795-0

Ohnishi Y, Kameyama S, Onaka H, Horinouchi S (1999) The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus*: identification of a target gene of the A-factor receptor. Mol Microbiol 34:102–111. doi:10.1046/j.1365-2958.1999.01579.x

Ohnishi Y, Yamazaki H, Kato J, Tomono A, Horinouchi S (2005) AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. Biosci Biotechnol Biochem 69:431–439. doi:10.1271/bbb.69.431

Oliynyk M et al (2003) Analysis of the biosynthetic gene cluster for the polyether antibiotic monensin in *Streptomyces cinnamonensis* and evidence for the role of *monB* and *monC* genes in oxidative cyclization. Mol Microbiol 49:1179–1190. doi:10.1046/j.1365-2958.2003.03571.x

Onaka H, Ando N, Nihira T, Yamada Y, Beppu T, Horinouchi S (1995) Cloning and characterization of the A-factor receptor gene from *Streptomyces griseus*. J Bacteriol 177:6083–6092

Onaka H, Nakagawa T, Horinouchi S (1998) Involvement of two A-factor receptor homologues in *Streptomyces coelicolor* A3(2) in the regulation of secondary metabolism and morphogenesis. Mol Microbiol 28:743–753. doi:10.1046/j.1365-2958.1998.00832.x

Ostash B et al (2011) Identification and characterization of the *Streptomyces globisporus* 1912 regulatory gene *lndYR* that affects sporulation and antibiotic production. Microbiology 157:1240–1249. doi:10.1099/mic.0.045088-0

Otten SL, Ferguson J, Hutchinson CR (1995) Regulation of daunorubicin production in *Streptomyces peucetius* by the *dnrR2* locus. J Bacteriol 177:1216–1224

Otten SL, Olano C, Hutchinson CR (2000) The *dnrO* gene encodes a DNA-binding protein that regulates daunorubicin production in *Streptomyces peucetius* by controlling expression of the *dnrN* pseudo response regulator gene. Microbiology 146:1457–1468. doi:10.1099/00221287-146-6-1457

Ou X, Zhang B, Zhang L, Zhao G, Ding X (2009) Characterization of *rrdA*, a TetR family protein gene involved in the regulation of secondary metabolism in *Streptomyces coelicolor*. Appl Environ Microbiol 75:2158–2165. doi:10.1128/aem.02209-08

Palaniappan N, Ayers S, Gupta S, Habib ES, Reynolds KA (2006) Production of hygromycin A Analogs in *Streptomyces hygroscopicus* NRRL 2388 through identification and manipulation of the biosynthetic gene cluster. Chem Biol 13:753–764. doi:10.1016/j.chembiol.2006.05.013

Parajuli N, Moon YH (2002) Analysis of doxorubicin biosynthetic gene cluster and intensive study of regulatory system of *Streptomyces peucetious* ATCC 27952. Theor Appl Chem Eng 8:3909–3912

Parajuli N, Viet HT, Ishida K, Tong HT, Lee HC, Liou K, Sohng JK (2005) Identification and characterization of the *afsR* homologue regulatory gene from *Streptomyces peucetius* ATCC 27952. Res Microbiol 156:707–712. doi:10.1016/j.resmic.2005.03.005

Pérez-Llarena FJ, Liras P, Rodríguez-García A, Martín JF (1997) A regulatory gene (*ccaR*) required for cephamycin and clavulanic acid production in *Streptomyces clavuligerus*: amplification results in overproduction of both beta-lactam compounds. J Bacteriol 179:2053–2059

Pérez-Redondo R, Rodríguez-García A, Martín JF, Liras P (1998) The *claR* gene of *Streptomyces clavuligerus*, encoding a LysR-type regulatory protein controlling clavulanic acid biosynthesis, is linked to the clavulanate-9-aldehyde reductase (*car*) gene. Gene 211:311–321. doi:10.1016/S0378-1119(98)00106-1

Pullan ST, Chandra G, Bibb MJ, Merrick M (2011) Genome-wide analysis of the role of GlnR in *Streptomyces venezuelae* provides new insights into global nitrogen regulation in actinomycetes. BMC Genom 12:1–14. doi:10.1186/1471-2164-12-175

Pulsawat N, Kitani S, Nihira T (2007) Characterization of biosynthetic gene cluster for the production of virginiamycin M, a streptogramin type A antibiotic, in *Streptomyces virginiae*. Gene 393:31–42. doi:10.1016/j.gene.2006.12.035

Pulsawat N, Kitani S, Fukushima E, Nihira T (2009) Hierarchical control of virginiamycin production in *Streptomyces virginiae* by three pathway-specific regulators: VmsS, VmsT and VmsR. Microbiology 155:1250–1259. doi:10.1099/mic.0.022467-0

Räty K, Kantola J, Hautala A, Hakala J, Ylihonko K, Mäntsälä P (2002) Cloning and characterization of *Streptomyces galilaeus* aclacinomycins polyketide synthase (PKS) cluster. Gene 293:115–122. doi:10.1016/S0378-1119(02)00699-6

Rehakova A, Novakova R, Feckova L, Mingyar E, Kormanec J (2013) A gene determining a new member of the SARP family contributes to transcription of genes for the synthesis of the angucycline polyketide auricin in *Streptomyces aureofaciens* CCM 3239. FEMS Microbiol Lett 346:45–55

Retzlaff L, Distler J (1995) The regulator of streptomycin gene expression, StrR, of *Streptomyces griseus* is a DNA binding activator protein with multiple recognition sites. Mol Microbiol 18:151–162. doi:10.1111/j.1365-2958.1995.mmi_18010151.x

Rodríguez M, Núñez LE, Braña AF, Méndez C, Salas JA, Blanco G (2008) Identification of transcriptional activators for thienamycin and cephamycin C biosynthetic genes within the thienamycin gene cluster from *Streptomyces cattleya*. Mol Microbiol 69:633–645. doi:10.1111/j.1365-2958.2008.06312.x

Rodríguez H, Rico S, Díaz M, Santamaría RI (2013) Two-component systems in *Streptomyces*: key regulators of antibiotic complex pathways. Microb Cell Fact 12:127. doi:10.1186/1475-2859-12-127

Romero-Rodríguez A, Robledo-Casados I, Sánchez S (2015) An overview on transcriptional regulators in *Streptomyces*. Biochem Biophys Acta 1849:1017–1039. doi:10.1016/j.bbagrm.2015.06.007

Sato K, Nihira T, Sakuda S, Yanagimoto M, Yamada Y (1989) Isolation and structure of a new butyrolactone autoregulator from *Streptomyces* sp. FRI-5. J Ferment Bioeng 68:170–173. doi:10.1016/0922-338X(89)90131-1

Sekurova ON et al (2004) In vivo analysis of the regulatory genes in the nystatin biosynthetic gene cluster of *Streptomyces noursei* ATCC 11455 reveals their differential control over antibiotic biosynthesis. J Bacteriol 186:1345–1354. doi:10.1128/jb.186.5.1345-1354.2004

Sheeler NL, MacMillan SV, Nodwell JR (2005) Biochemical activities of the *absA* two-component system of *Streptomyces coelicolor*. J Bacteriol 187:687–696. doi:10.1128/jb.187.2.687-696.2005

Sola-Landa A, Moura RS, Martín JF (2003) The two-component PhoR-PhoP system controls both primary metabolism and secondary metabolite biosynthesis in *Streptomyces lividans*. Proc Natl Acad Sci USA A100:6133–6138. doi:10.1073/pnas.0931429100

Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Annu Rev Biochem 69:183–215. doi:10.1146/annurev.biochem.69.1.183

Stratigopoulos G, Cundliffe E (2002) Expression analysis of the tylosin-biosynthetic gene cluster: pivotal regulatory role of the *tylQ* product. Chem Biol 9:71–78. doi:10.1016/S1074-5521(01)00095-3

Suzuki T, Mochizuki S, Yamamoto S, Arakawa K, Kinashi H (2010) Regulation of lankamycin biosynthesis in *Streptomyces rochei* by two SARP genes, *srrY* and *srrZ*. Biosci Biotechnol Biochem 74:819–827. doi:10.1271/bbb.90927

Takano E, Gramajo HC, Strauch E, Andres N, White J, Bibb MJ (1992) Transcriptional regulation of the *redD* transcriptional activator gene accounts for growth-phase-dependent production

of the antibiotic undecylprodigiosin in *Streptomyces coelicolor* A3(2). Mol Microbiol 6:2797–2804. doi:10.1111/j.1365-2958.1992.tb01459.x

Takano E et al (2005) A bacterial hormone (the SCB1) directly controls the expression of a pathway-specific regulatory gene in the cryptic type I polyketide biosynthetic gene cluster of *Streptomyces coelicolor*. Mol Microbiol 56:465–479. doi:10.1111/j.1365-2958.2005.04543.x

Tanaka A, Takano Y, Ohnishi Y, Horinouchi S (2007) AfsR recruits RNA polymerase to the *afsS* promoter: a model for transcriptional activation by SARPs. J Mol Biol 369:322–333. doi:10.1016/j.jmb.2007.02.096

Wang L et al (2009a) Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator. Proc Natl Acad Sci USA 106:8617–8622. doi:10.1073/pnas.0900592106

Wang W, Shu D, Chen L, Jiang W, Lu Y (2009b) Cross-talk between an orphan response regulator and a noncognate histidine kinase in *Streptomyces coelicolor*. FEMS Microbiol Lett 294:150–156. doi:10.1111/j.1574-6968.2009.01563.x

Wilson DJ, Xue Y, Reynolds KA, Sherman DH (2001) Characterization and analysis of the PikD regulatory factor in the pikromycin biosynthetic pathway of *Streptomyces venezuelae*. J Bacteriol 183:3468–3475. doi:10.1128/jb.183.11.3468-3475.2001

Xie PF, Sheng Y, Ito T, Mahmud T (2012) Transcriptional regulation and increased production of asukamycin in engineered *Streptomyces nodosus* subsp. asukaensis strains. Appl Microbiol Biotechnol 96:451–460. doi:10.1007/s00253-012-4084-2

Xue Y, Zhao L, Liu HW, Sherman DH (1998) A gene cluster for macrolide antibiotic biosynthesis in *Streptomyces venezuelae*: architecture of metabolic diversity. Proc Natl Acad Sci USA 95:12111–12116. doi:10.1073/pnas.95.21.12111

Yamada Y, Sugamura K, Kondo K, Yanagimoto M, Okada H (1987) The structure of inducing factors for virginiamycin production in *Streptomyces virginiae*. J Antibiot 40:496–504. doi:10.7164/antibiotics.40.496

Yang K, Han L, He J, Wang L, Vining LC (2001) A repressor-response regulator gene pair controlling jadomycin B production in *Streptomyces venezuelae* ISP5230. Gene 279:165–173. doi:10.1016/S0378-1119(01)00723-5

Yepes A, Rico S, Rodríguez-García A, Santamaría RI, Díaz M (2011) Novel two-component systems implied in antibiotic production in *Streptomyces coelicolor*. PLoS ONE 6:e19980. doi:10.1371/journal.pone.0019980

Yoo YJ, Hwang JY, Shin HL (2015) Characterization of negative regulatory genes for the biosynthesis of rapamycin in *Streptomyces rapamycinicus* and its application for improved production. Microbiol Biotechnol 42:125–135

Yu Z, Wang Q, Deng Z, Tao M (2006) Activation of silent antibiotic synthesis in *Streptomyces lividans* by disruption of a negative regulator *nsdA*, a gene conserved in *Streptomyces* Chinese. J Biotechnol 22:757–762

Yu Q, Du A, Liu T, Deng Z, He X (2012) The biosynthesis of the polyether antibiotic nanchangmycin is controlled by two pathway-specific transcriptional activators. Arch Microbiol 194:415–426. doi:10.1007/s00203-011-0768-8

Zhang Y, Zou Z, Niu G, Tan H (2013) *jadR** and *jadR2* act synergistically to repress jadomycin biosynthesis. Sci China Life Sci 56:584–590. doi:10.1007/s11427-013-4508-y

Zhu D, He X, Zhou X, Deng Z (2005) Expression of the *melC* operon in several streptomyces strains is positively regulated by AdpA, an AraC family transcriptional regulator involved in morphological development in *Streptomyces coelicolor*. J Bacteriol 187:3180–3187. doi:10.1128/jb.187.9.3180-3187.2005

Zou Z, Du D, Zhang Y, Zhang J, Niu G, Tan H (2014) A γ-butyrolactone-sensing activator/repressor, JadR3, controls a regulatory mini-network for jadomycin biosynthesis. Mol Microbiol 94:490–505. doi:10.1111/mmi.12752