The Journal of Antibiotics (2009) 62, 669–673

www.nature.com/ja

ORIGINAL ARTICLE

Activation of secondary metabolite–biosynthetic gene clusters by generating *rsmG* mutations in *Streptomyces griseus*

Yukinori Tanaka[1,2], Shinji Tokuyama[2] and Kozo Ochi[1]

Unlike other *Streptomyces* spp., the streptomycin producer *Streptomyces griseus* IFO13189 shows emergence of a small fraction of *rsmG* and *rpsL* mutants among spontaneous low- or high-level streptomycin-resistant mutants. *rsmG*, but not *rpsL*, mutants showed greater ability (two- to threefold) to produce streptomycin, accompanied by enhanced transcription of *metK* and *strR*, together with streptomycin biosynthetic genes, such as *strB1*, *strD* and *strF*, thus underlying the observed increase in streptomycin production in the *rsmG* mutants. Moreover, *rsmG* mutation was effective for activating the 'silent' or poorly expressed secondary metabolite–biosynthetic genes present in *S. griseus*.
The Journal of Antibiotics (2009) **62,** 669–673; doi:10.1038/ja.2009.97; published online 9 October 2009

Keywords: *rsmG*; silent genes; *Streptomyces griseus*; streptomycin resistance

INTRODUCTION

Actinomycetes produce a variety of natural products that are of major importance in the pharmaceutical industry. More than 50% of all anti-infective and anticancer compounds developed over the past 25 years have been natural products or derivatives thereof.[1] In the past several years, the complete genome sequences of many organisms have been reported. The information from these genome projects indicated that *Streptomyces coelicolor*, *S. avermitilis* and *S. griseus* have 20, 25 and 34 clusters of genes involved in secondary metabolism, respectively, but only a few secondary metabolites are known in each case.[2–4] Recently, we described a practical method for increasing antibiotic production in bacteria by modulating ribosomal components (ribosomal proteins or rRNA), specifically by generating mutations conferring drug resistance, such as streptomycin resistance.[5–7] This approach, called 'ribosome engineering',[8] has several advantages including the ability to screen for drug resistance mutations by simple selection on drug-containing plates, even if the mutation frequency is extremely low (for example, $<10^{-10}$), and has been shown to be effective for improving the industrial strains, which had been bred to produce large amount of antibiotics.[9,10] *S. griseus* is a filamentous, soil-living, Gram-positive bacteria, which produces an aminoglycoside antibiotic, streptomycin, and is characterized by the presence of a streptomycin self-resistance gene, *aphD*, which encodes streptomycin-6-phosphotransferase. Here, we showed that *rsmG* and *rpsL* (conferring low and high levels of resistance to streptomycin, respectively) mutations can be generated in *S. griseus*, although this organism already possesses a relatively high level of self-resistance to this antibiotic produced by the organism itself. The effects of *rsmG* and *rpsL* mutations on streptomycin production were examined, along with those of many other 'silent' secondary metabolite–biosynthetic genes present in this organism.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Streptomyces griseus IFO13189, a prototrophic streptomycin-producing wild-type strain, was used as the parental strain. Spontaneous streptomycin-resistant mutants were obtained as colonies that grew within 5–7 days after spores were spread on glucose–yeast extract–malt extract (GYM) agar[11] containing various concentrations of streptomycin. Mutations in *rpsL* or *rsmG* genes were determined by DNA sequencing using the primers listed in Table 1. Cultivation was performed at 30 °C (for preculture) or 25 °C (for main culture) using SPY medium[11] with rotary shaking at 220 r.p.m.

Assay for streptomycin and determination of minimum inhibitory concentrations

Streptomycin was determined by bioassay using *Bacillus subtilis* ATCC6633 as the test organism. Minimum inhibitory concentrations (MICs) were determined by spotting spore solutions ($\sim 10^6$) onto streptomycin-containing GYM plates, followed by incubation for 5 days at 30 °C.

Total RNA preparation

Total cellular RNA was prepared using Isogen reagent (Nippon Gene, Toyama, Japan). Each pellet was resuspended in 1 ml of Isogen reagent and incubated at 50 °C for 10 min. After cooling, 0.2 ml of chloroform was added. Each sample was mixed well by vortexing and centrifuged at 16 000×*g* for 10 min, and 0.6 ml of the aqueous phase (top layer) was transferred to a clean tube. To each test tube, 0.4 ml of chloroform was added and each sample was mixed by repeated inversions and centrifuged at 16 000×*g* for 10 min. A portion (0.6 ml) of the aqueous phase (top layer) was transferred to a clean tube. The RNA in each

[1]National Food Research Institute, Tsukuba, Ibaraki, Japan and [2]Faculty of Agriculture, Department of Applied Biological Chemistry, Shizuoka University, Shizuoka, Japan
Correspondence: Dr K Ochi, National Food Research Institute, 2–1–12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan.
E-mail: kochi@affrc.go.jp
Received 14 July 2009; revised 10 September 2009; accepted 18 September 2009; published online 9 October 2009

Table 1 The primers used in this study

Purpose	*Primer*	*Oligonucleotide sequence (5′ → 3′)*	*Reference*
PCR and sequencing for *rpsL*	rpsL-F1	CGACACACCCGACCGCGTGGG	Tanaka *et al.*[6]
	rpsL-R1	CGGGTCGATGATGACCGGGCGCTTC	
PCR and sequencing for *rsmG*	rsmG-F2	CGGAAGGACGGTCCCCGTG	Tanaka *et al.*[6]
	rsmG-R2	GAGCGACGTTTCACGTGAAACACGATGC	
Real-time qPCR for *hrdB*	SGR1701 hrdB F	GAAGGTCATCGAGGTCCAGAAG	Ohnishi *et al.*[4]
	SGR1701 hrdB R	GTGGCGGAGCTTCGACATC	
Real-time qPCR for *adpA*	adpA-77F	TTGCCGTGCTGCTGTTCA	This study
	adpA-130R	AAACGGAGAGCGGGATGGA	
Real-time qPCR for *metK*	metK-F2	CAACCTCGTGCGCAACAA	This study
	metK-R2	CACAGGAAGCACCGTCGAA	
Real-time qPCR for *strR*	SGR5931 strR F	AATTATCCGCCGTGACAATGG	Ohnishi *et al.*[4]
	SGR5931 strR R	GGATGGGTCTCCAGGACAC	
Real-time qPCR for *strB1*	strB1-F2	ACTACGAGAGCCAGGAGCAGAT	This study
	strB1-R2	TGACTCCGAGCTTGGTCAACT	
Real-time qPCR for *strD*	strD-F2	ACCCACACGTCTGCGAAAC	This study
	strD-R2	TGGCCTCCAGCCCATAGA	
Real-time qPCR for *strF*	StrF-F2	GACTACGACAAGGTGCACGACTA	This study
	StrF-R2	AAGCGGATGTTCTCGATGGT	
Real-time qPCR for SGR 281	281-802F	CGCACGATCTGGAAGAACCT	This study
	281-874R	GGTAGTAGAGGCGATCGACGTAGA	
Real-time qPCR for SGR 443	443-643F	GTGCAGTCCCGCGAGATG	This study
	443-727R	GGTAGCGCCGTTCGTTGTC	
Real-time qPCR for SGR 593	593-44F	GTTCCTTGCCTGACCTGAATG	This study
	593-101R	CGCGAGAGAAGCGGATCA	
Real-time qPCR for SGR 604	604-15F	GCCCTACTACGAACTGCGTCAT	This study
	604-78R	GTAGACGTTGCCGACCAGGTT	
Real-time qPCR for SGR 811	811-F825	CCTGCCCTACCTGATGTTTCC	This study
	811-R899	CAGTTCTGTTCGGTGAACCATTC	
Real-time qPCR for SGR 896	896-F702	GGAGTGCCGCGAGATCTTC	This study
	896-R770	TCGAGGACGGCGATGCTT	
Real-time qPCR for SGR 962	962-551F	TCCCCCTCAACATCTACGACTT	This study
	962-627R	GCGTTTCGCGGAGACGAT	
Real-time qPCR for SGR 2079	2079-190F	GCCACACAGGCCCATCTC	This study
	2079-245R	ACCAGGAAGGCCCAGAAAA	
Real-time qPCR for SGR 2488	2488-501F	GTCCGGCTCGATCGTCAAC	This study
	2488-582R	GTGCTTGGCCGCGACGTA	
Real-time qPCR for SGR 2594	2594-825F	CGTCTTCGGCATGGTCATG	This study
	2594-889R	ATCTGCTCGACGGGTTCCA	
Real-time qPCR for SGR 3267	3267-F945	CGTCGTCACGCTCTGGAA	This study
	3267-R1007	TCCTCAGGACGGTCGAACAC	
Real-time qPCR for SGR 4413	4413-140F	TCGCCGGGTACTTCTTCATC	This study
	4413-199R	TGAGGCGCAGCCGTACGT	
Real-time qPCR for SGR 5295	5295-F189	CAACGACTACCTGGGCATGA	This study
	5295-R253	GCAGGGTGGAGGCGATCT	
Real-time qPCR for SGR 6072	6072-F27	CACCGTCCTGGAGTACTTCGA	This study
	6072-R99	GTCGGTGGCGAACAGCTT	
Real-time qPCR for SGR 6178	6178-F372	CCGGGCTCCGGTGATC	This study
	6178-R424	CGTCATCGCCCCTCAGATG	
Real-time qPCR for SGR 6367	6367-F142	GCGTTCACGTCCGTTTCC	This study
	6367-R198	GCTGCGGGCGACACA	
Real-time qPCR for SGR 6717	6717-864F	CGCGCAGTTCATCATGGAA	This study
	6717-922R	TCATCACGTACTTGGGCATCTC	
Real-time qPCR for SGR 6780	6780-F381	CGGCGTCTCCGAGCAGAT	This study
	6780-R457	CGTTGTGGTTGGCGATGAC	

Abbreviation: qPCR, quantitative PCR.

tube was precipitated with an equal volume of isopropanol, and rinsed with 70% ethanol. The Isogen reagent treatment was repeated once more to prepare pure RNA and finally suspended with an appropriate volume of diethylpyrocarbonate-treated water.

Transcriptional analysis by real-time quantitative PCR

The total RNAs were prepared as described above. Contaminating DNA was removed by incubating each total RNA sample (1 μg) with 1 U of DNAse I (Invitrogen, Carlsbad, CA, USA) for 15 min at 25 °C. Reverse transcription

Table 2 Characterization of streptomycin resistance mutations of *Streptomyces griseus*

Streptomycin concentration ($\mu g\,ml^{-1}$) used for selection	*Mutation in*[a]		*Amino acid substitution*	*Frequency of mutants with the same mutation*	*Minimum inhibitory concentration to streptomycin ($\mu g\,ml^{-1}$)*[b]	*Streptomycin productivity ($\mu g\,ml^{-1}$)*[c]	*Designation of mutant*
	rsmG	*rpsL*					
—[d]					70	65 ± 6	
70	Δ73G–76T		Frameshift	1/86	300	118 ± 27	KO-1057
	Insertion of AGCTC at 88		Frameshift	1/86	300	117 ± 27	KO-1058
	173T→C		Leu58→Pro	1/86	300	154 ± 30	KO-1059
	268G→C		Gly90→Arg	1/86	300	192 ± 21	KO-1060
	Insertion of CCA at 277		Insertion of Pro at 93	1/86	300	124 ± 17	KO-1061
	280G→C		Ala94→Pro	1/86	300	144 ± 18	KO-1062
	331C→T		Arg111→Trp	1/86	300	124 ± 17	KO-1063
	332G→A		Arg111→Gln	1/86	300	130 ± 5	KO-1064
	Δ389T–398G		Frameshift	1/86	300	139 ± 33	KO-1065
	Insertion of G at 465		Frameshift	1/86	300	121 ± 18	KO-1066
	500A501G→C		Frameshift	1/86	300	151 ± 8	KO-1067
	Insertion of G at 585		Frameshift	1/86	300	164 ± 36	KO-1069
	Δ623T		Frameshift	1/86	300	125 ± 4	KO-1070
	ND[e]		ND	72/86	—[f]	—[f]	
100	Δ260G–291G		Frameshift	1/30	300	140 ± 8	KO-1050
	Δ389T–398G		Frameshift	1/30	300	162 ± 17	KO-1051
	ND		ND	28/30	—[f]	—[f]	
500		262A→G	Lys88→Glu	4/59	2000	57 ± 13	KO-1052 ~ 1055
		ND	ND	55/59	—[f]	—[f]	
1000		Insertion of GGCGTGCGT at 250	Insertion of Gly, Val, Arg at 84	1/20	3000	29 ± 7	KO-1056
		ND	ND	19/20	—[f]	—[f]	
2000		ND	ND	9/9	—[f]	—[f]	

Abbreviation: GYM, glucose–yeast extract–malt extract.
[a]Numbered from the start codon of the open reading frame of *S. griseus*.
[b]Determined after 5 days of incubation on GYM medium.
[c]Strains were grown in SPY medium at 25 °C for 3 days.
[d]Wild-type strain.
[e]Mutations were not detected in either rsmG or rpsL gene.
[f]Not determined.

reaction was carried out using a high-capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The samples were diluted with an appropriate volume of water and analyzed using the 7300 real-time quantitative PCR (qPCR) system and Power SYBR Green PCR master mix (Applied Biosystems). Each transcriptional assay was normalized to the corresponding transcriptional level of the *hrdB* gene encoding the principal sigma factor. Primers used for real-time qPCR are listed in Table 1. All reactions were performed under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for denaturing, 30 s at 60 °C for annealing and 30 s at 72 °C for extension.

RESULTS AND DISCUSSION

Isolation and characterization of streptomycin-resistant mutants

In various actinomycetes, high-level resistance to streptomycin is often due to mutations in *rpsL*, which encodes the ribosomal protein S12, whereas low-level resistance is due to mutations in *rsmG*, which encodes a 16S rRNA methyltransferase.[5,6] Unlike other *Streptomyces* spp. (MIC to streptomycin, 0.2–2 $\mu g\,ml^{-1}$), the streptomycin producer *S. griseus* IFO13189 showed a relatively high level of resistance (MIC=70 $\mu g\,ml^{-1}$). We isolated a total of 204 resistant mutant strains that had developed spontaneously on GYM agar plates containing various concentrations (70–2000 $\mu g\,ml^{-1}$) of streptomycin. Of these mutants, 15 had mutations in the *rsmG* gene and were characterized by the frequent appearance of deletion or insertion mutations that resulted in frameshift, and five carried mutations in *rpsL*, including the K88E mutation and novel insertion mutation (Table 2). These results were in contrast with those of previous studies in other *Streptomyces* spp, in which >50% of resistant strains were due to mutations in *rpsL* or *rsmG*.[6] This observation suggested that *S. griseus* harbors a wide range of resistance systems, in addition to inactivation of streptomycin by streptomycin-6-phosphotransferase, to survive in the presence of its own antibiotic, streptomycin. The *rsmG* mutants showed impaired ability to form aerial mycelia, and were somewhat deficient in sporulation.

We reported previously that, in the *rsmG* mutant background, *rpsL* mutants with high-level streptomycin resistance emerge at a frequency 200-fold greater than that in the wild-type strain of *S. coelicolor*, and this elevated frequency in the emergence of high-level streptomycin resistance was facilitated by a mutation pattern in *rpsL* more varied than that obtained by selection of the wild-type strain.[5,6] Similarly,

Table 3 Genes of *Streptomyces griseus* IFO13189 analyzed in this study

Gene[a]	*Product*	*Secondary metabolite biosynthetic gene cluster*
metK	*S*-adenosylmethionine synthetase	—[b]
adpA	Transcriptional regulator	—[b]
strR	Streptomycin biosynthesis operon regulator	Streptomycin
strB1	Scyllo-inosamine-4-phosphate amidinotransferase	Streptomycin
strD	Putative glucose-1-phosphate thymidylyltransferase	Streptomycin
strF	StrF protein	Streptomycin
SGR281	Hypothetical protein	PKS–NRPS hybrid (SGR278–SGR283)
SGR443	Putative ABC transporter ATPase and permease component	NRPS for siderophore (SGR443–SGR455)
SGR593	Hypothetical protein	NRPS (SGR574–SGR593)
SGR604	Putative enediyne biosynthesis protein	Enediyne PKS (SGR604–SGR611)
SGR811	Putative oxidoreductase	PKS-NRPS hybrid (SGR810–SGR815)
SGR896	Putative O-methyltransferase	NRPS (SGR895–SGR901)
SGR962	Putative squalene–hopene cyclase	Hopanoid (SGR962–SGR966)
SGR2079	Putative terpene cyclase	Terpene (SGR2079)
SGR2488	Putative dehydrogenase	Type I PKS, NRPS (SGR2482–SGR2489)
SGR2594	Putative integral membrane ion antiporter	NRPS (SGR2586–SGR2598)
SGR3267	Putative cytochrome P450	Type II PKS, NRPS (SGR3239–SGR3288)
SGR4413	Putative lantibiotic biosynthesis protein	Lantibiotic (SGR4408–SGR4421)
SGR5295	5-Aminolevulinate synthase	Unknown (SGR5285–SGR5295)
SGR6072	Putative ketosteroid isomerase	Type I PKS (SGR6071–SGR6083)
SGR6178	Putative thioesterase	Type I PKS (SGR6177–SGR6183)
SGR6367	Putative oxidoreductase	Type I PKS (SGR6360–SGR6387)
SGR6717	Putative ABC transporter ATPase and permease component	NRPS for siderophore (SGR6709–SGR6717)
SGR6780	Putative malonyl-CoA: ACP transacylase	Type I PKS, PKS–NRPS hybrid (SGR6776–SGR6786)

[a]Gene names are obtained from the reference Ohnishi *et al.*[4]
[b]These are not the secondary metabolite biosynthetic gene cluster.

S. griseus rsmG mutant, KO-1050, generated the mutants resistant to high-level streptomycin (1000 μg ml^{-1}) at high frequency. However, no *rpsL* mutants were detected among the 30 resistant mutants tested, again characterizing the specific feature of *S. griseus*.

Effects of *rsmG* and *rpsL* mutations on streptomycin production

Mutations in *rsmG* were effective in enhancing streptomycin production, leading to a two- to threefold increase in streptomycin production, whereas *rpsL* K88E mutation and *rpsL* insertion mutation were not effective (Table 2). In addition, the mutants with no mutations in either *rsmG* or *rpsL* (that is, ND mutants) showed the impaired ability to produce streptomycin, as examined with 12 ND mutants (data not shown). In *S. coelicolor*, enhanced expression of the *metK* gene encoding *S*-adenosylmethionine synthetase corresponds to the enhanced production of streptomycin and actinorhodin caused by *rsmG* mutations.[5,12] Moreover, addition of *S*-adenosylmethionine causes overproduction of streptomycin in *S. griseus*, accompanied by enhanced transcription of *adpA* and *strR*.[13,14] We analyzed transcription of *metK*, *adpA* and *strR*, together with several genes involved in biosynthesis of streptomycin (*strB1*, *strF* and *strD*) (Table 3).

As expected, the *rsmG* mutant, but not the *rpsL* K88E mutant, exhibited elevated levels of *metK*, *strR*, *strB1*, *strF* and *strD* expression compared with the wild-type strain at late growth phase (36 h) (Figure 1), thus underlying the enhanced production of streptomycin in the *rsmG* mutant. StrR is a regulator of the streptomycin biosynthesis operon[15] and AdpA acts as a central transcriptional regulator in the A-factor regulatory cascade of *S. griseus*.[16] Although the elevated levels of *metK* and *strR* expression account well for the marked enhancement of biosynthetic gene expression (*strB1*, *strD* and *strF*), it is not yet clear why *adpA* gene expression was not activated in the *rsmG* mutant.

Effects of *rsmG* mutation on transcription of secondary metabolite–biosynthetic gene clusters

A recent study in our laboratory indicated that certain mutations in *rpoB* (encoding RNA polymerase β-subunit) or *rpsL* genes can activate 'silent' genes of actinomycetes, leading to the discovery of novel antibacterial agents.[17] The activation of silent genes by generating *rpoB* H437D or *rpoB* H437L mutations in *Streptomyces* sp. 631689 was attributed, at least partly, to the increased affinity of the mutant RNA polymerase for promoters.[17] Thus, we analyzed the expression of secondary metabolite–biosynthetic gene clusters in the *S. griseus rsmG* mutant, KO-1050. A total of 18 genes belonging to 18 secondary metabolite–biosynthetic gene clusters (Table 3) were subjected to transcriptional analysis by real-time qPCR, and the results indicated that the levels of expression of these genes are quite low (1/10–1/1000 compared with that of *strB1*), thus representing silent or poorly expressed genes. Strikingly, analysis of cells harvested at late growth phase (36 h) indicated that the *rsmG* mutation affected not only streptomycin biosynthesis but also the expression of other secondary metabolite–biosynthetic genes, although the effects were not dramatic (Figure 2). Enhancement of expression was pronounced in SGR3267 (putative cytochrome P450 involved in Type II polyketide synthases or non-ribosomal peptide synthetases) and SGR962 (putative squalene–hopene cyclase involved in hopanoid synthesis). By contrast, the *rpsL* K88E mutant, KO-1052, did not show any enhanced expression of those genes (data not shown).

CONCLUDING REMARKS

We showed that *rsmG* mutation is effective not only for enhancement of streptomycin production but also for activation of silent or poorly expressed genes in *S. griseus*. Although a biochemical approach (for example, measurement of *in vitro* protein synthesis activity) was not

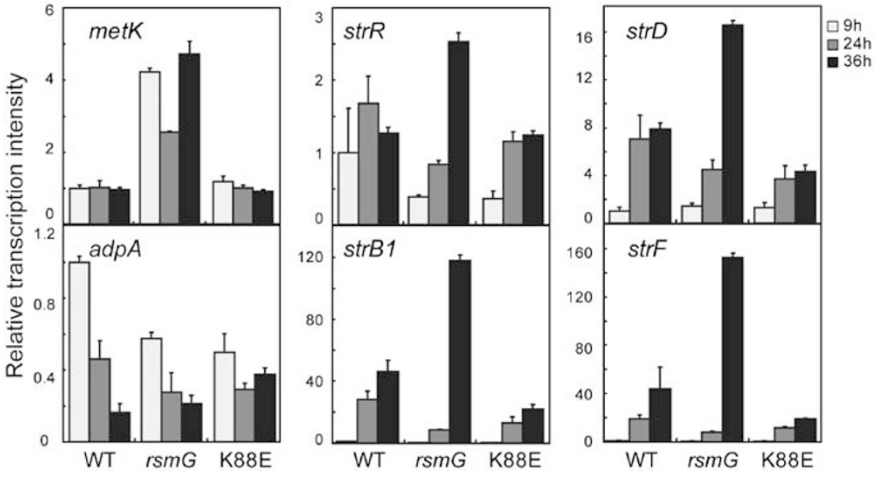

Figure 1 Transcriptional analysis of *adpA*, *metK*, *strR*, *strB1*, *strD* and *strF* by real-time quantitative PCR (qPCR). The RNAs were extracted from cells of wild-type (13189), *rsmG* (KO-1050) and *rpsL* K88E (KO-1052) mutant strains grown to early growth phase (9 h), mid growth phase (24 h) or late growth phase (36 h) in SPY medium. Total RNA preparation and real-time qPCR were performed as described in Materials and Methods. The error bars indicate the standard deviations of the means of three or more samples.

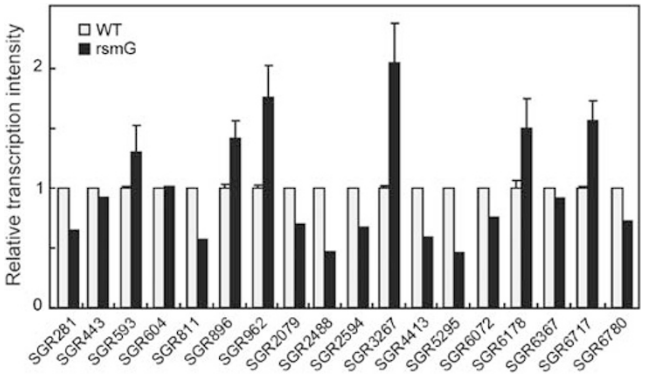

Figure 2 Transcriptional analysis of the genes involved in the secondary metabolite–biosynthetic gene clusters. The RNAs were extracted from cells of wild-type (13189) and *rsmG* (KO-1050) mutant strains grown to late growth phase (36 h). Total RNA preparation and real-time quantitative PCR was performed as described in Materials and Methods. Error bars indicate the standard deviations of the means of three or more samples.

employed in the present study, it is possible that the expression of pathway-specific regulatory genes is governed by higher-order regulatory proteins and expression of such higher-order regulatory proteins may be significantly affected under conditions associated with enhanced *metK* expression in mutants. The present method, together with other methods reported recently,[6,7,17–20] may be useful for activating silent genes, eventually leading to the discovery of novel antibacterial agents.

ACKNOWLEDGEMENTS

This work was supported by grants to KO (Effective Promotion of Joint Research of Special Coordination Funds) from the Ministry of Education, Culture, Sports and Technology of the Japanese Government.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **70,** 461–477 (2007).
2 Bentley, S. D. *et al.* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417,** 141–147 (2002).
3 Ikeda, H. *et al.* Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat. Biotechnol.* **21,** 526–531 (2003).
4 Ohnishi, Y. *et al.* Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. *J. Bacteriol.* **190,** 4050–4060 (2008).
5 Nishimura, K., Hosaka, T., Tokuyama, S., Okamoto, S. & Ochi, K. Mutations in *rsmG*, encoding a 16S rRNA methyltransferase, result in low-level streptomycin resistance and antibiotic overproduction in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **189,** 3876–3883 (2007).
6 Tanaka, Y. *et al.* Antibiotic overproduction by *rpsL* and *rsmG* mutants of various actinomycetes. *Appl. Environ. Microbiol.* **75,** 4919–4922 (2009).
7 Wang, G., Hosaka, T. & Ochi, K. Dramatic activation of antibiotic production in *Streptomyces coelicolor* by cumulative drug resistance mutations. *Appl. Environ. Microbiol.* **74,** 2834–2840 (2008).
8 Ochi, K. From microbial differentiation to ribosome engineering. *Biosci. Biotechnol. Biochem.* **71,** 1373–1386 (2007).
9 Tamehiro, N. *et al.* Innovative approach for improvement of an antibiotic-overproducing industrial strain of *Streptomyces albus*. *Appl. Environ. Microbiol.* **69,** 6412–6417 (2003).
10 Beltrametti, F., Rossi, R., Selva, E. & Marinelli, F. Antibiotic production improvement in the rare actinomycete *Planobispora rosea* by selection of mutants resistant to the aminoglycosides streptomycin and gentamycin and to rifamycin. *J. Ind. Microbiol. Biotechnol.* **33,** 283–288 (2006).
11 Ochi, K. Metabolic initiation of differentiation and secondary metabolism by *Streptomyces griseus*: significance of the stringent response (ppGpp) and GTP content in relation to A factor. *J. Bacteriol.* **169,** 3608–3616 (1987).
12 Okamoto, S., Lezhava, A., Hosaka, T., Okamoto Hosoya, Y. & Ochi, K. Enhanced expression of S-adenosylmethionine synthetase causes overproduction of actinorhodin in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **185,** 601–609 (2003).
13 Saito, N., Kurosawa, K., Xu, J., Okamoto, S. & Ochi, K. Effect of S-adenosylmethionine on antibiotic production in *Streptomyces griseus* and *Streptomyces griseoflavus*. *Actinomycetologica* **17,** 47–49 (2003).
14 Shin, S. K., Xu, D., Kwon, H. J. & Suh, J. W. S-adenosylmethionine activates *adpA* transcription and promotes streptomycin biosynthesis in *Streptomyces griseus*. *FEMS Microbiol. Lett.* **259,** 53–59 (2006).
15 Retzlaff, L. & Distler, J. 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 (1995).
16 Ohnishi, Y., Yamazaki, H., Kato, J. Y., Tomono, A. & Horinouchi, S. 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 (2005).
17 Hosaka, T. *et al.* Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. *Nat. Biotechnol.* **27,** 462–464 (2009).
18 Rigali, S. *et al.* Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*. *EMBO Rep.* **9,** 670–675 (2008).
19 Carata, E. *et al.* Phenotypes and gene expression profiles of *Saccharopolyspora erythraea* rifampicin-resistant (*rif*) mutants affected in erythromycin production. *Microb. Cell. Fact.* **8,** 18 (2009).
20 Tala, A. *et al.* Activation of dormant bacterial genes by *Nonomuraea* sp. strain ATCC 39727 mutant-type RNA polymerase. *J. Bacteriol.* **191,** 805–814 (2009).