J Ind Microbiol Biotechnol (2012) 39:805–811
DOI 10.1007/s10295-011-1083-8

SHORT COMMUNICATION

Minimal polyketide pathway expression in an actinorhodin cluster-deleted and regulation-stimulated *Streptomyces coelicolor*

Han-Na Lee · Hye-Jin Kim · Pil Kim · Heung-Shick Lee · Eung-Soo Kim

Received: 5 November 2011 / Accepted: 31 December 2011 / Published online: 18 January 2012

Abstract Along with traditional random mutagenesis-driven strain improvement, cloning and heterologous expression of *Streptomyces* secondary metabolite gene clusters have become an attractive complementary approach to increase its production titer, of which regulation is typically under tight control via complex multiple regulatory networks present in a metabolite low-producing wild-type strain. In this study, we generated a polyketide non-producing strain by deleting the entire actinorhodin cluster from the chromosome of a previously generated *S. coelicolor* mutant strain, which was shown to stimulate actinorhodin biosynthesis through deletion of two antibiotic downregulators as well as a polyketide precursor flux downregulator (Kim et al. in Appl Environ Microbiol 77:1872–1877, 2011). Using this engineered *S. coelicolor* mutant strain as a surrogate host, a model minimal polyketide pathway for aloesaponarin II, an actinorhodin shunt product, was cloned in a high-copy conjugative plasmid, followed by functional pathway expression and quantitative metabolite analysis. Aloesaponarin II production was detected only in the presence of a pathway-specific regulatory gene, *actII-ORF4*, and its production level was the highest in the actinorhodin cluster-deleted and downregulator-deleted mutant strain, implying that this engineered polyketide pathway-free and regulation-optimized *S. coelicolor* mutant strain could be used as a general surrogate host for efficient expression of indigenous or foreign polyketide pathways derived from diverse actinomycetes in nature.

Keywords *Streptomyces* · Polyketide expression · Antibiotic regulation stimulation

Electronic supplementary material The online version of this article (doi:10.1007/s10295-011-1083-8) contains supplementary material, which is available to authorized users.

H.-N. Lee · H.-J. Kim · E.-S. Kim (✉)
Department of Biological Engineering, Inha University, Incheon 402-751, Korea
e-mail: eungsoo@inha.ac.kr

P. Kim
Department of Biotechnology, The Catholic University of Korea, Bucheon 420-743, Gyeonggi, Korea

H.-S. Lee
Department of Biotechnology and Bioinformatics, Korea University, Jochiwon 339-700, Chungnam, Korea

Introduction

It is well documented that the bacterial genus *Streptomyces* produces the majority of known microbial-origin pharmaceutically valuable secondary metabolites [1, 17]. The expression of these *Streptomyces* secondary metabolites are tightly regulated by multiple complex regulatory networks; however, many of these pathways were found to be present in silent or less-expressed strains in nature [19, 23]. For the last several decades, optimization of *Streptomyces* culture and recursive random mutagenesis has been adopted as one of the most widely practiced strategies for utilizing these potentially valuable but tightly regulated secondary metabolites [6, 18, 21]. In addition, understanding the complex molecular-level regulatory networks and their practical application are becoming equally crucial steps for successful *Streptomyces* strain improvement.

As an alternative and complementary strategy for *Streptomyces*-driven novel metabolite isolation, functional expression of the target secondary metabolite pathway in a *Streptomyces* heterologous host has been applied. Several

Table 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference
Strains		
S. coelicolor M145	Wild type	[11]
S. coelicolor ESK101	Actinorhodin cluster deleted *S. coelicolor* M145	This study
S. coelicolor ESK104	Actinorhodin cluster deleted *S. coelicolor* M145 with Δ*wblA*Δ1712Δ5426	This study
Plasmids		
pKC1139	*E. coli-Streptomyces* shuttle vector; *aac(3)IV lacZα oriT*$_{RK2}$	[4]
pKC-ACT	pKC1139 derivative containing 2.7 kb and 2.9 kb homologous flaking regions of actinorhodin cluster	This study
pWHM3	*E. coli-Streptomyces* shuttle vector; *lacZα bla tsr*	[11]
pMMBL004	pWHM3 derivative containing *Xba*I digested apr/oriT fragment from pIJ773	This study
pAloeII-1	pMMBL004 containing the *actI, actIII, actVII* and *actIV* loci	This study
pAloeII-2	pMMBL004 containing the *actI, actIII, actVII, actIV* loci and *actII-orf4*	This study

pathways of pharmaceutically valuable secondary metabolites were successfully expressed in relatively well-characterized and genetically amenable *Streptomyces* surrogate hosts, including *S. coelicolor*, *S. lividans*, and *S. avermitilis* [7, 8, 20, 22, 24]. Recently, the genomes of these strains were further engineered to maximize production of foreign metabolites by deleting the endogenous secondary metabolic gene clusters and/or preventing the diversion of precursors into competing secondary metabolic pathways [2, 16]. In addition, a genome-minimized *Streptomyces* industrial mutant strain was also successfully used for foreign metabolite pathway expression as a heterologous expression host [14], implying that the development of versatile and efficient *Streptomyces* surrogate hosts is critical for future actinomycetes natural product development.

Recently, we isolated previously unidentified regulatory genes involved in antibiotic biosynthetic regulation, including two global antibiotic downregulatory genes (a *whiB*-like protein A gene, *wblA*, and a *tetR* family transcriptional regulatory gene, SCO1712), using reverse engineering approaches via comparative transcriptomics between a wild-type and a metabolite-overproducing industrial mutant strain [10, 15]. Sequential targeted gene disruption of these two antibiotic downregulator genes as well as polyketide precursor flux downregulating SCO5426 led to a significant synergistic increase in the production of a type II polyketide antibiotic actinorhodin (ACT) in this triple knock-out *S. coelicolor* mutant [13]. Here, we deleted the entire ACT cluster from the chromosome of this triple knock-out *S. coelicolor* mutant strain to generate a polyketide pathway-deleted and secondary metabolite regulation-stimulated *S. coelicolor* mutant strain. Using this *S. coelicolor* mutant as a surrogate host, a model minimal type II polyketide named aloesaponarin II derived from the ACT shunt pathway could be functionally overexpressed using a conjugative high-copy shuttle plasmid. These results demonstrated that this engineered polyketide pathway-free and regulation-optimized *S. coelicolor* strain might become a general surrogate host for efficient expression of indigenous or foreign polyketide pathways derived from diverse actinomycetes genome mining.

Materials and methods

Bacterial strains and growth condition

The strains and plasmids used in this study are listed in Table 1. *S. coelicolor* M145 was cultured in a modified R5 liquid medium [11] at 30°C for 5 days. *S. coelicolor* strains harboring pKC-ACT were cultured at 37°C. All *Streptomyces* strains were cultured with 50 μg/ml of apramycin or 50 μg/ml thiostrepton when needed. *E. coli* strains were cultured at 37°C in Luria broth or on Luria agar, supplemented with the appropriate antibiotics when needed (50 μg/ml ampicillin; 50 μg/ml kanamycin; 50 μg/ml apramycin; 50 μg/ml chloramphenicol).

Construction of ACT cluster-deleted mutants

A gene replacement plasmid pKC-ACT, which was derived from pKC1139 and contained an apramycin resistance marker, was constructed for deletion of the ACT cluster. The plasmid pKC-ACT was constructed by cloning 2.7-kb and 2.9-kb fragments homologous to the SCO5070-5072

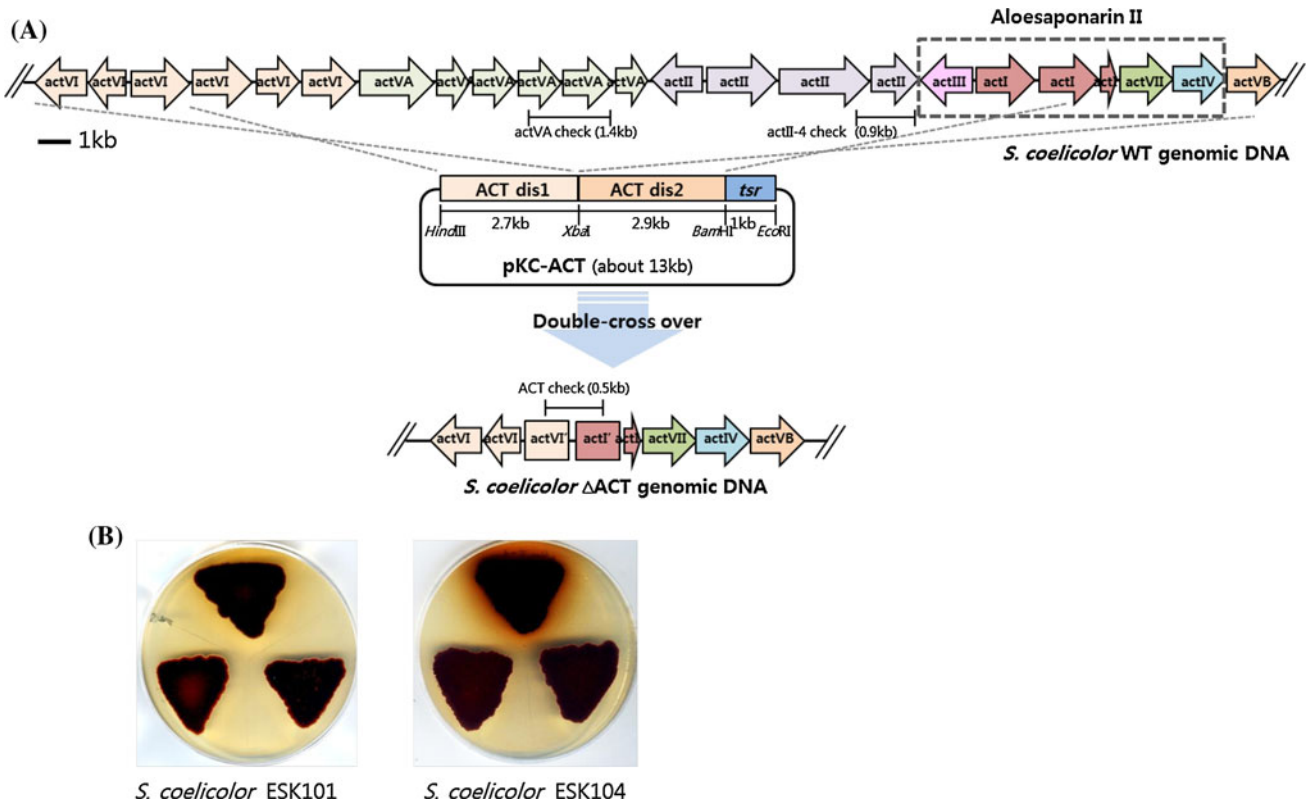

Fig. 1 **a** Schematic representation of the actinorhodin (ACT) biosynthetic gene cluster used to generate the ACT cluster deleted strain. *Dashed box* represents genes for aloesaponarin II biosynthesis. *tsr*: thiostrepton^{R}. **b** The phenotypes of ACT cluster-deleted *streptomyces* strains on the modified R5 plates for 5 days. *Left plate* shows *S. coelicolor* ESK101 harboring pMMBL004 (*bottom left*), pAloeII-1 (*bottom right*), and pAloeII-2 (*top*). *Right plate* shows *S. coelicolor* Δ*wblA*Δ1712Δ5426 ESK104 harboring pMMBL004 (*bottom left*), pAloeII-1 (*bottom right*), and pAloeII-2 (*top*)

and SCO5088-5092 regions of the ACT cluster, and cloning the 1 kb *tsr* fragment. Each of the fragments was individually amplified using the polymerase chain reaction (PCR) (BioRad, USA) with ACT dis1 primer pairs, ACT dis2 primer pairs, and *tsr* primer pairs. All PCR primer sequences used in this study are summarized in supplemental table 1. All fragments were sequenced and introduced into pKC1139 digested with *Hind*III-*Eco*RI. The resulting plasmid was introduced into *S. coelicolor* M145 and *S. coelicolor* triple knock-out (Δ*wblA*Δ1712Δ5426) mutant strain by conjugation. Thiostrepton resistance mutants were selected and sub-cultured four times in modified R5 broth media with 50 μg/ml thiostrepton at 37°C. The collected spores from modified R5 plates containing thiostrepton were cultured in modified R5 broth without thiostrepton at 37°C. After four repeats of subculture, several double crossover mutants were identified based on the phenotypes of thiostrepton sensitivity and loss of ACT blue pigment production. The correct deletion of the ACT gene cluster from the mutant chromosome was further verified via PCR amplification of the deleted-junction and its sequencing analysis.

Construction of aloesaponarinII expression vector

The constructed pMMBL004, derived from pWHM3, was used for aloesaponarin II expression (Table 1). It was constructed for conjugation by ligation of a *Xba*I digested apr^{R}/oriT fragment from pIJ773 [9] using the *E. coli-Streptomyces* shuttle vector pWHM3 (Fig. 2a). The pAloeII-1 vector contains an additional 8.8-kb *Pst*I fragment including *actI*, *actIII*, *actVII*, and *actIV* loci isolated from pANT12 [12]. The *actII*-orf4 fragment containing its own promoter was PCR-amplified from the *S. coelicolor* M145 chromosome, and its sequence was verified. The *Eco*RI digested *actII*-orf4 fragment was introduced into the pAloeII-1 vector, which was designated pAloeII-2. All constructed vectors were introduced into *Streptomyces* by conjugation, and thiostrepton and apramycin were used for selection.

HPLC analyses of aloesaponarin II and its carboxylic form

Twenty milliliters of the cultures in triple-baffled flasks was acidified to pH 3 with 1 M citric acid and then

extracted with an equal volume of 10% MeOH/$CHCl_3$ [12]. The resulting emulsion was re-extracted with the same solvent. The 5-day-old solid cultures of ESK101 and ESK104 grown on two 30-ml modified R5 agar plates containing apramycin and thiostrepton were ground with 100 ml of ddH_2O using a blender. Then 250 ml of 10% MeOH/$CHCl_3$ was added to the acidified emulsion and reextracted with the same solvent. The total extracts were dissolved in 1 ml of MeOH. Aloesaponarin II compounds were visualized on thin-layer chromatography (TLC) plates (Whatman, Inc., USA), which were developed with a solvent system containing cyclohexane and ethyl acetate (1:1) as described previously [3, 12]. The compounds on the TLC plates were visualized based on their normal pigmentation and by their fluorescence under long-wave UV irradiation at 365 nm. The purified aloesaponarin II and its acid form on the TLC plate were also confirmed by ESI/MS (Varian, USA) and used as standards for HPLC analysis. The concentration of aloesaponarin II and its acid form (ε_{410} = 3,100) [5] were measured using a UV–visible spectrophotometer (Shimazu, Japan). The culture extracts were then subjected to analysis using an HPLC system (Shimazu, Japan) for aloesaponarin II quantification with a C_{18} column (5 µm, 250 mm × 4.6 mm, Agilent, USA). For HPLC analysis, an isocratic mobile phase buffer of 65% methanol, 30% water, and 5% glacial acetic acid (final pH 2.5) was used, and the sample was monitored at 410 nm.

Results and discussion

Deletion of the actinorhodin gene cluster from *S. coelicolor* wild-type and mutant strains

To generate actinorhodin (ACT) non-producing *S. coelicolor* strains, the entire ACT biosynthetic gene clusters were deleted from the *S. coelicolor* wild-type M145 and the ACT-overproducing triple knock-out (Δ*wblA*Δ1712Δ5426) *S. coelicolor* mutant using a temperature-sensitive suicide vector pKC1139, respectively (Fig. 1a). As previously reported, the triple knock-out (Δ*wblA*Δ1712Δ5426) *S. coelicolor* mutant displayed about fourfold higher ACT-specific productivity compared with the wild-type strain [13]. As described in Materials and methods, *S. coelicolor* ΔACT (ESK101) and *S. coelicolor* ΔACTΔ*wblA*Δ1712Δ5426 (ESK104) strains were precisely engineered, which was confirmed by PCR analyses and direct sequencing of the junction fragment. The expected PCR-amplified size of the 0.5-kb band was observed both in ESK 101 and ESK104 strains, but not in the wild-type and triple knock-out strains (Suppl. Fig. 1). Moreover, the junction fragments amplified by PCR were further verified to be correct by direct sequencing in both strains (Supp. Fig. 2). As expected, both *S. coelicolor* mutant strains failed to produce a deep-blue pigment ACT, while the bald phenotype observed in the triple knock-out mutant strain was similar to the ESK101 strain (Fig. 1b). Based on both genotypic and phenotypic characterization, the entire ACT biosynthetic gene clusters were shown

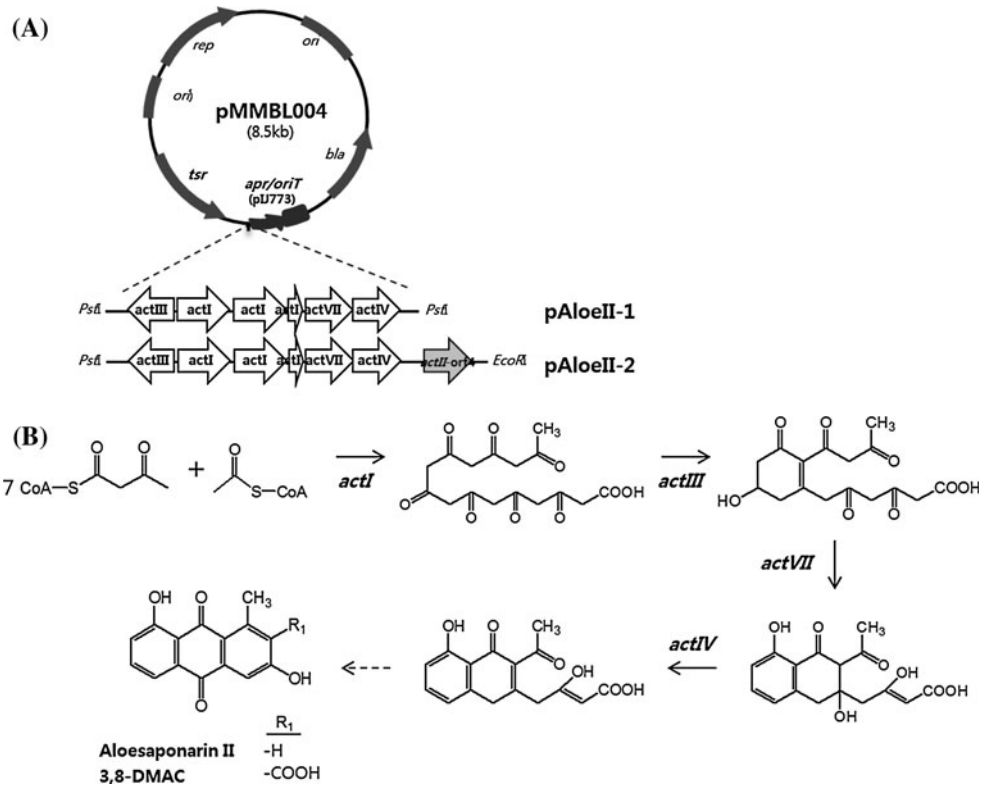

Fig. 2 **a** Plasmid map of the pMMBL004, pAloeII-1 (pMMBL004 containing *Pst*I fragment), pAloeII-2 (pAloeII-1 containing *actII-orf4*). *rep*: replicon; *ori*: origin of replication; *bla*: β-lactamase^R; *tsr*: thiostrepton^R; *apr*: apramycin^R; *oriT*: origin of transfer. **b** Schematic represent of the the aloesaponarin II biosynthetic pathway. *Solid* and *dotted arrows* represent presumed enzymatic and spontaneous steps, respectively. 3,8-DMAC: 3,8-dihydroxy-1-methyl-anthraquinone-2-carboxylic acid

to be successfully deleted from the chromosomes of *S. coelicolor* ESK101 and ESK104 strains.

Cloning and functional expression of the minimal polyketide aloesaponarin II pathway

It was previously reported that an 8.8-kb *Pst*I fragment containing the *actI*, *actIII*, and *actVII* loci from the ACT biosynthetic cluster cloned into the high-copy number *Streptomyces* vector pIJ350 led to a highly fluorescent ACT shunt product called aloesaponarin II in a polyketide non-producing *S. parvulus* [12], suggesting that the six ORFs present in these loci are the minimal number of type II polyketide genes needed to generate a stable and detectable shunt product (Fig. 2a). As shown in Fig. 2b, aloesaponarin II was derived from one acetyl-CoA starter unit and seven malonyl-CoA extender units. In order to express this minimal polyketide aloesaponarin II biosynthetic pathway in *S. coelicolor* ESK101 and ESK104 strains, an 8.8-kb *Pst*I fragment containing the *actI*, *actIII*, and *actVII* loci was sub-cloned into a *E. coli-Streptomyces* conjugative high-copy number shuttle plasmid pMMBL004, resulting in pAloeII-1 (Fig. 2a). The pMMBL004 was constructed from the *Streptomcyes* high-copy number plasmid, pWHM3, after insertion of the conjugative *Xba*I fragment containing $apr^R/oriT$. The main advantage of using the pMMBL004 system is the ability to transfer the target plasmid via conjugation directly from the *E. coli* donor to the *Streptomyces* recipient without unexpected mutations related to secondary metabolite productivity during protoplast formation and regeneration.

S. coelicolor ESK101/pAloeII-1 and ESK104/pAloeII-1 strains were grown in either liquid or solid culture, but all the strains failed to produce any detectable amount of aloesaponarin II (Figs. 1b, 3), presumably because of the lack of a pathway-specific activator in the cluster. Since the bi-directional promoter located in the cloned aloesaponarin II biosynthetic cluster was shown to be activated only in the presence of *actII-ORF4*, an ACT pathway-specific activator gene, we decided to clone the 1-kb *Eco*RI fragment containing the entire *actII-ORF4* gene and its promoter into the pAloeII-1 construct, resulting in pAloeII-2 (Fig. 2a). Unlike the strains containing pAloeII-1, the *S. coelicolor* ESK101/pAloeII-2 and ESK104/pAloeII-2 strains grown on solid plates started to produce typical dark-yellowish aloesaponarin II pigments (Fig. 1b). To further verify

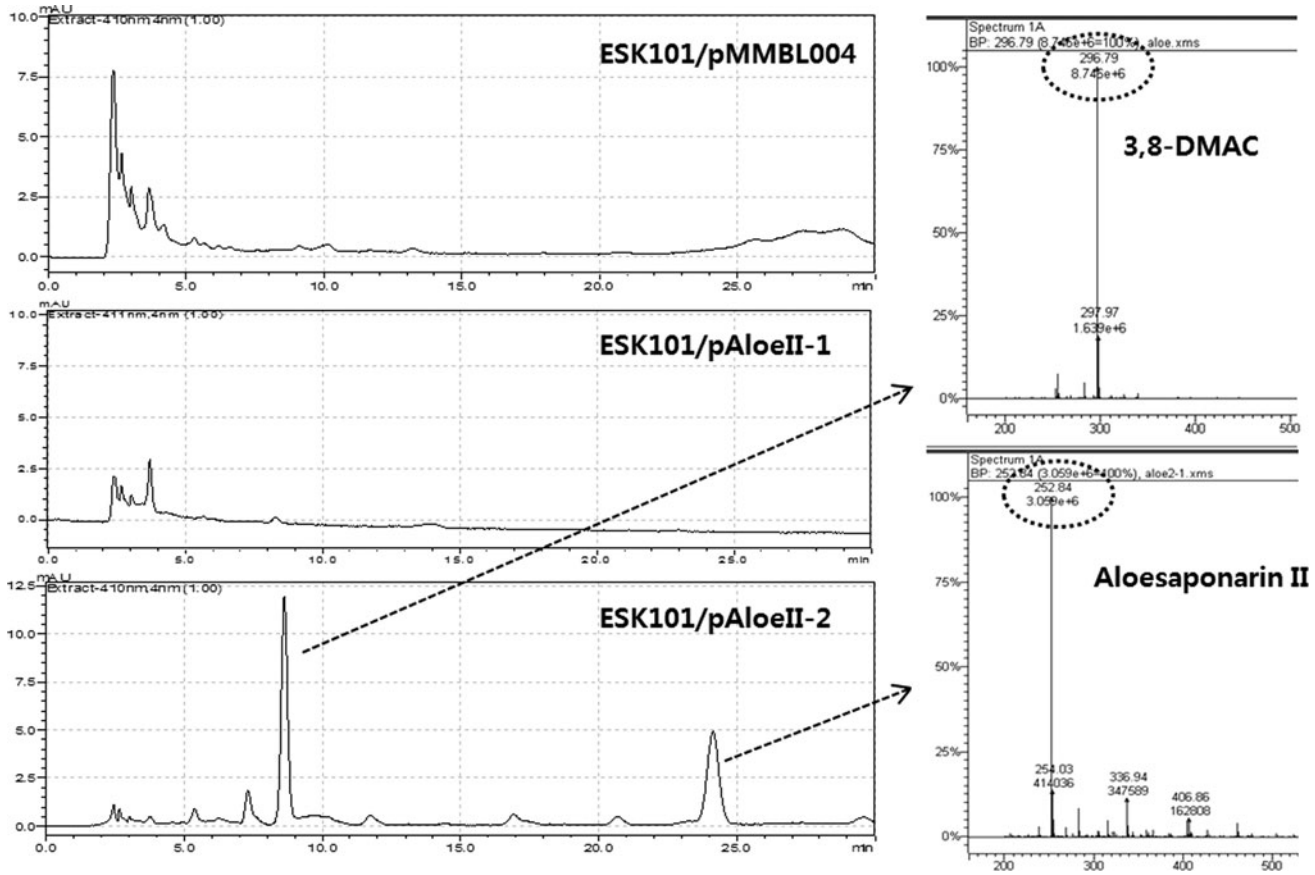

Fig. 3 HPLC chromatograms of the *S. coelicolor* ESK101 containing pMMBL004 (*top*), pAloeII-1 (*middle*), and pAloeII-2 (*bottom*) with ESI–MS analysis data

Table 2 Productions of aloesaponarin II and 3,8-DMAC in recombinant *S. coelicolor* strains

Strains	Total of 3,8-DMAC and aloesaponarin II (mg/g)
Liquid culture[a]	
ESK101/pMMBL004	ND[c]
ESK104/pMMBL004	ND
ESK101/pAloeII-1	ND
ESK104/pAloeII-1	ND
ESK101/pAloeII-2	3.76
ESK104/pAloeII-2	7.45
Solid culture[b]	
ESK101/pAloeII-2	3.67
ESK104/pAloeII-2	22.15

[a] Average values of triplet liquid cultures

[b] Average values of two-independent plate cultures

[c] *ND* not detected

aloesaponarin II production, total plate-grown extracts from *S. coelicolor* ESK101/pAloeII-2 and ESK104/pAloeII-2 were applied to thin-layer chromatography (TLC) as well as HPLC. Highly fluorescent orange spots were detected only in the *S. coelicolor* ESK101/pAloeII-2 and ESK104/pAloeII-2 strains, but not in the *S. coelicolor* ESK101/pMMBL004, ESK104/pMMBL004, ESK101/pAloeII-1, or ESK104/pAloeII-1 strains (data not shown), of which the TLC Rf value was similar to the previously reported TLC Rf value of aloesaponarin II [3]. The same extracts exhibited two distinctive peaks in the reverse phase HPLC column assay (Fig. 3). The molecular weights of aloesaponarin II and its carboxylated form, 3,8-DMAC (3,8-hydroxy-1-methyl-anthraquinone-2-carboxylic acid), were determined to be 254 and 298, respectively, in the ESI–MS analyses (Fig. 3), suggesting that the minimal polyketide pathway with its own pathway-specific regulatory gene should be sufficient to produce the target polyketide compounds in our engineered *S. coelicolor* strains.

Comparative production of aloesaponarin II in engineered *S. coelicolor* strains

The *S. coelicolor* ESK104/pAloeII-2 and ESK104/pAloeII-2 strains were cultured in liquid media to produce aloesaponarin II along with the *S. coelicolor* strains containing pMMBL004 or pAloeII-1. While all the strains exhibited comparable growth patterns in 5-day liquid cultures, the *S. coelicolor* ESK104/pAloeII-2 strain exhibited the highest aloesaponarin II-specific productivity (7.4 mg/g DCW), which was about twofold higher than that of the *S. coelicolor* ESK101/pAloeII-2 (3.7 mg/g DCW) (Table 2). These results suggest that the deletion of polyketide downregulating genes such as *wblA*/SCO1712/SCO5426 led to significant stimulation of aloesaponarin II biosynthesis. Since *S. coelicolor* ESK104/pAloeII-1 failed to produce any detectable amounts of aloesaponarin II, a pathway-specific regulatory gene such as *actII-ORF4* is essential for *Streptomyces* heterologous expression. Moreover, the 5-day modified R5 plate culture extract from *S. coelicolor* ESK104/pAloeII-2, which was showing much darker-yellowish pigments, exhibited sixfold higher aloesaponarin II-specific productivity (22.2 mg/g DCW) than that obtained from the liquid culture extract of the same ESK101/pAloeII-2 strain (Fig. 1b; Table 2), implying that a functional polyketide pathway could be better expressed in an engineered *Streptomyces* strain grown under a solid culture condition. In conclusion, a *wblA*/SCO1712/SCO5426-deleted *S. coelicolor* mutant strain without an ACT biosynthetic gene cluster was successfully generated for functional expression of a model minimal type II polyketide aloesaponarin II. The results of this work suggest that this engineered polyketide pathway-free and regulation-optimized *S. coelicolor* strain might be used as a general surrogate host to maximize functional expression of indigenous or foreign polyketide pathways derived from diverse actinomycete genome mining.

Acknowledgments This work was supported by the Korean Systems Biology Program (MEST 2011-0002151) as well as the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST 2011-0027683).

References

1. Andexer JN, Kendrew SG, Nur-e-Alam M, Lazos O, Foster TA, Zimmermann AS, Warneck TD, Suthar D, Coates NJ, Koehn FE, Skotnicki JS, Carter GT, Gregory MA, Martin CJ, Moss SJ, Leadlay PF, Wilkinson B (2011) Biosynthesis of the immunosuppressants FK506, FK520, and rapamycin involves a previously undescribed family of enzymes acting on chorismate. Proc Natl Acad Sci USA 108:4776–4781
2. Baltz RH (2010) *Streptomyces* and *Saccharopolyspora* hosts for heterologous expression of secondary metabolite gene clusters. J Ind Microbiol Biotechnol 37:759–772
3. Bartel PL, Zhu CB, Lampel JS, Dosch DC, Connors NC, Strohl WR, Beale JM Jr, Floss HG (1990) Biosynthesis of anthraquinones by interspecies cloning of actinorhodin biosynthesis genes in *streptomycetes*: clarification of actinorhodin gene functions. J Bacteriol 172:4816–4826
4. Bierman M, Logan R, O'Brien K, Seno ET, Rao RN, Schoner BE (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces spp*. Gene 116:43–49
5. Bystrykh LV, Herrema JK, Kruizinga W, Kellogg RM (1997) 5-Hydroxyaloesaponarin II, a minor blue pigment in an actinorhodin-negative mutant of *Streptomyces coelicolor* A3(2). Biotechnol Appl Biochem 26:195–201
6. Chen X, Tang L, Li S, Liao L, Zhang J, Mao Z (2011) Optimization of medium for enhancement of epsilon-poly-L-lysine production by *Streptomyces* sp. M-Z18 with glycerol as carbon source. Bioresour Technol 102:1727–1732
7. Flinspach K, Westrich L, Kaysser L, Siebenberg S, Gomez-Escribano JP, Bibb M, Gust B, Heide L (2010) Heterologous

expression of the biosynthetic gene clusters of coumermycin A(1), clorobiocin and caprazamycins in genetically modified *Streptomyces coelicolor* strains. Biopolymers 93:823–832
8. Gomez-Escribano JP, Bibb MJ (2011) Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. Microb Biotechnol 4:207–215
9. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc Natl Acad Sci USA 100:1541–1546
10. Kang SH, Huang J, Lee HN, Hur YA, Cohen SN, Kim ES (2007) Interspecies DNA microarray analysis identifies WblA as a pleiotropic down-regulator of antibiotic biosynthesis in *Streptomyces*. J Bacteriol 189:4315–4319
11. Kieser T, Bibb MJ, Chater KF, Buttner MJ, Hopwood DA (2000) Practical *Streptomyces* genetics: a laboratory manual. John Innes Foundation, Norwich
12. Kim ES, Cramer KD, Shreve AL, Sherman DH (1995) Heterologous expression of an engineered biosynthetic pathway: functional dissection of type II polyketide synthase components in *Streptomyces* species. J Bacteriol 177:1202–1207
13. Kim SH, Lee HN, Kim HJ, Kim ES (2011) Transcriptome analysis of an antibiotic downregulator mutant and synergistic Actinorhodin stimulation via disruption of a precursor flux regulator in *Streptomyces coelicolor*. Appl Environ Microbiol 77:1872–1877
14. Komatsu M, Uchiyama T, Omura S, Cane DE, Ikeda H (2010) Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. Proc Natl Acad Sci USA 107:2646–2651
15. Lee HN, Huang J, Im JH, Kim SH, Noh JH, Cohen SN, Kim ES (2010) Putative TetR family transcriptional regulator SCO1712 encodes an antibiotic downregulator in *Streptomyces coelicolor*. Appl Environ Microbiol 76:3039–3043
16. Martinez A, Kolvek SJ, Yip CL, Hopke J, Brown KA, MacNeil IA, Osburne MS (2004) Genetically modified bacterial strains and novel bacterial artificial chromosome shuttle vectors for constructing environmental libraries and detecting heterologous natural products in multiple expression hosts. Appl Environ Microbiol 70:2452–2463
17. Niraula NP, Kim SH, Sohng JK, Kim ES (2010) Biotechnological doxorubicin production: pathway and regulation engineering of strains for enhanced production. Appl Microbiol Biotechnol 87:1187–1194
18. Olano C, Lombó F, Méndez C, Salas JA (2008) Improving production of bioactive secondary metabolites in actinomycetes by metabolic engineering. Metab Eng 10:281–292
19. Onaka H, Mori Y, Igarashi Y, Furumai T (2011) Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces* species. Appl Environ Microbiol 77:400–406
20. Wang XJ, Wang JJ, Wang JD, Zhang J, Xu MD, Xiang WS (2011) Two new doramectin analogs from *Streptomyces avermitilis* NEAU1069: fermentation, isolation and structure elucidation. J Antibiot (Tokyo) 64:591–594
21. Wu X, Yang D, Zhu X, Feng Z, Lv Z, Zhang Y, Shen B, Xu Z (2010) Iso-migrastatin titer improvement in the engineered *Streptomyces lividans* SB11002 strain by optimization of fermentation conditions. Biotechnol Bioprocess Eng 15:664–669
22. Young TS, Walsh CT (2011) Identification of the thiazolyl peptide GE37468 gene cluster from *Streptomyces* ATCC 55365 and heterologous expression in Streptomyces lividans. Proc Natl Acad Sci USA 108:13053–13058
23. Zerikly M, Challis GL (2009) Strategies for the discovery of new natural products by genome mining. Chembiochem 10:625–633
24. Zhu Y, Wang L, Du Y, Wang S, Yu T, Hong B (2011) Heterologous expression of human interleukin-6 in *Streptomyces lividans* TK24 using novel secretory expression vectors. Biotechnol Lett 33:253–261