Partial Characterization and Functional Expression of Omics-driven Potential Regulatory Genes for Antibiotics-Overproduction in *Streptomyces* species
Partial Characterization and Functional Expression of Omics-driven Potential Regulatory Genes for Antibiotics-Overproduction in Streptomyces species

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**Abstract**

Doxorubicin (DOX) is a highly valuable anticancer drug belonging to the anthracycline family, which is generated by a high G+C gram-positive soil bacterium, *Streptomyces peucetius*. It has been well documented that the biosynthesis of most antibiotics are tightly regulated at complicated genetic levels, thus leading to very low productivity in wild-type strains. To screen novel potential regulatory genes involved in antibiotic overproduction, wild type and overproducing strains such as actinorhodin (ACT)–producing *S. lividans* and DOX–producing *S. peucetius* were generated and compared via omics–driven strategies. Using 2-D gel electrophoresis and MALDI–TOF analyses between the wild type and the ACT–overproducing *S. lividans*, several proteomics guided targets were identified. Comparative transcriptomes were also analyzed between the wild type and the DOX–overproducing *S. peucetius via S. coelicolor* cDNA microarrays. We found several potential regulatory genes involved in antibiotics–overproduction. To show the biological significance of the omics–driven potential target genes in Streptomyces species, these genes were functionally expressed and partially characterized both in *S. coelicolor* and *S. peucetius*. Further analysis revealed that expression of some of these genes inhibited antibiotics production. The identification and manipulation of these cryptic regulatory genes described herein may result in the maximization of the genetic productivity potentials in *Streptomyces species*.

Key word: *Streptomyces*, Antibiotic, Omics
요 약

Doxorubicin은 *Streptomyces peucetius*에 의해 생합성되는 매우 강력한 항암제 중의 하나이다. 현재까지 doxorubicin을 비롯한 방선균 유래 2차 대사산물의 생산성 향상을 위해 주로 random mutation 방법이 상용되어 왔으나, 이러한 방법은 분자 유전학적 수준에서 2차 대사산물의 고생산 mechanism이 무엇인지를 알 수 없기 때문에 또 다른 균주의 개발을 위해서는 같은 방법을 반복하여 야 한다는 단점을 가지고 있다. 따라서 이러한 단점을 극복하기 위해서 omics 활용기술을 통한 기존에 밝혀지지 않은 2차 대사산물의 생산에 관여하는 후보 유전자들을 선별하고 이렇게 선별된 유전자들을 실제 방선균에서 발현시킴으로써 2차 대사산물의 생산에 관여하는 key regulator를 찾아야 한다.

따라서 본 연구에서는 항생제인 actinorhodin 저 생산균주인 *S. lividans* TK21과 antibiotic 생합성을 향상시키는 유전자인 afsR2가 chromosom에 integration되어 actinorhodin이 고생산되는 균주인 *S. lividans* ESK206를 2D-gel과 MALDI-TOF를 이용하여 단백질 발현패턴을 비교분석해 보았고, doxorubicin 저 생산균주인 *S. peucetius* ATCC27952를 반복적인 mutation과 screening으로 doxorubicin 생산성을 증가시킨 *S. peucetius* BRdox과 cDNA chip을 이용하여 전사체 발현패턴의 차이를 비교 분석하여 보았다. 그중 항생제 고생산에 관여하는 가능성을 있는 여러개의 후보 유전자를 찾아냈다. 이러한 후보 유전자들의 방선균에서의 생물학적 특성을 확인하기 위해 *S. coelicolor* 와 *S. peucetius*에서 기능적인 발현을 시켜보았고, 이를 통해 각각의 유전자를 특정화 할 수 있었다. 추가적인 분석을 통해 실제로 항생제 생산에 영향을 미치는 몇 개의 유전자를 선별 할 수 있었다. 이러한 기준에 발현되지 않은 조절 유전자의 동정과 조작을 통해서 방선균 유래 항생제 생산을 극대화 시키는 계기가 될 수 있을 것이다.
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1. Introduction

1.1 *Streptomyces*

The bacterial genus *Streptomyces* is widely known for its ability to produce a variety of secondary metabolites, including products of medical importance such as antibiotics, antitumor agents, immunosuppressors, and enzyme inhibitors [1, 2, 3]. Production of most secondary metabolites by *Streptomyces* generally occurs during the stationary phase of cell growth with complicated mechanisms, and correlates temporally with the formation of aerial mycelium in cultures grown on the surface of solid media [1, 2, 4, 5, 6] (fig. 1). Thus far, this complex *Streptomyces* regulatory network has been partially identified. Several key regulatory genes have been revealed some of these affect only secondary metabolite production, while others pleiotropically affect both metabolite production and morphological differentiation, suggesting the presence of multiple regulatory systems [4, 7] (fig. 2).
Figure 1. Life cycle of *Streptomyces*. 
Figure 2. Secondary metabolites produced from *Streptomyces* species.
1.2 *afs*-gene family

The *afs*-gene family, which includes *afsR*, *afsK*, and *afsR2*, is one of several regulatory genes previously reported to affect the antibiotic biosynthetic pathways in *Streptomyces* spp. [7, 9, 10, 27, 29]. *afsR2* in *S. lividans*, which is also known as *afsS* in *S. coelicolor* [22], is located immediately 3 to *afsR*, and encodes a 63–amino–acid protein of which the function and mechanism might be related to the sigma factor protein [17]. It was reported that *afsR2* expression is physiologically regulated in *S. lividans*, and mRNA synthesis from a single chromosomal *afsR2* gene can be stimulated under specific growth conditions [15]. Moreover, the AfsR2–induced actinorhodin overproduction in both *S. coelicolor* and *S. lividans* was experimentally verified to be regulated via glycerol–inducible and glucose–repressible mechanism [15]. Recently, the wild–type *S. lividans*, which does not produces actinorhodin under typical growth conditions, was successfully transformed into the actinorhodin overproducing strain via a single chromosomal integration of *afsR2* [13]. This was followed by the comparative proteomics analyses aimed at isolating the putative AfsR2–target proteins involved in actinorhodin regulation [11]. This communication reports the functional expression of several putative AfsR2–target proteins identified from the comparative proteomics, one of the most powerful tools for analyzing the complex protein network systems [4, 5, 19, 20, 28], highlighting the biological significance of these putative AfsR2 target genes in *S. lividans* and *S. coelicolor*. 
1.3 Doxorubicin

Doxorubicin is a clinically very important anticancer drug that belongs to a structural family of type II polyketide compounds generated by a high G+C Gram-positive soil bacterium, *Streptomyces peucetius var. caesius* strain (1, 11, 18). Like the biosynthesis of other secondary metabolites in *Streptomyces species*, the synthesis of doxorubicin is believed to be tightly regulated, thereby limiting doxorubicin production in wild-type *S. peucetius* cultures (6, 12, 17). Traditional strain improvement via recursive random mutagenesis has been used to increase doxorubicin synthesis, although the molecular genetic basis underlying such enhanced production remains largely unknown (13, 14, 15, 18).

1.4 Omics-

Recently, "Omics"-guided technologies including cDNA microarrays have been applied for the identification of gene expression alterations associated with overproduction of secondary metabolites in industrial strains. While analysis of transcriptional changes in erythromycin-producing *Saccharopolysora erythreae* and
tylosin–producing *S. fradiae* using sequenced *S. coelicolor* cDNA microarrays (10) has revealed differences in the transcriptomes between the wild–type and the industrial over–producer, genes whose perturbation significantly affected productivity were not verified experimentally (10). In this brief communication, we report the identification of a previously–unknown down–regulator gene via comparisons of gene transcription profiles using DNA microarrays. Overexpression of this gene, *wblA*, which has 50% identity in a 64 amino acid overlap with the developmentally important *whiB* gene of *S. coelicolor*, inhibited the biosynthesis of doxorubicin in *S. peucetius* as well as the production of antibiotics in *S. coelicolor*, suggesting that *wblA* and its homologs act globally among streptomycetes as down–regulators of antibiotic biosynthesis.
2. **Materials & Methods**

2.1 **Bacterial strains, plasmids, and growth condition**

*Streptomyces lividans* TK21 and *Streptomyces coelicolor* M145 were cultured on R2YE agar and YEME liquid medium for isolation of the total DNA and protoplast transformation [15]. *E. coli* DH5α and the *Streptomyces* expression vector [16], pSE34[17], were used for the cloning and expression experiments according to standard molecular biology procedures.

2.2 **Cloning of omics-driven target genes**

Based on the publicly-available genome sequence information of *S. coelicolor* M145, each of the seventeen target genes was individually amplified using a polymerase chain reaction (PCR) [Idaho Technology, USA] with *BamH* -containing the forward primer and the *Hind*III-containing reverse primer. The PCR primer sequences are summarized in Table 1. The PCR conditions used for the high G+C DNA amplification program are as follows: denaturation at 96°C for 30 sec, annealing at 40°C for 30 sec, and extension at 72°C for 35 sec [14]. All seventeen PCR-amplified target genes include the putative upstream ribosome binding site, start codon, and stop codon sequences.
Each of the seventeen PCR-amplified target genes was then cloned into the pMD18-T vector [Takara, Japan], which was followed by complete sequence verification. The target genes were then subcloned into the *Streptomyces* expression vector pSE34 [17].

2.3 Functional expression of target genes in *S. lividans* TK21 and *S. coelicolor* M14

Each of the seventeen plasmids was introduced into *S. lividans* TK21 and *S. coelicolor* M145 individually using the polyethylene glycol (PEG)–mediated protoplast transformation method, followed by the thistrepton (tsr) selection method [15]. The level of actinorhodin production was determined by culturing each of *S. lividans* and *S. coelicolor* transformants containing the plasmid on a R2YE plate containing 50g/ml thistrepton at 30°C for 6 days.

2.4 Assay of antibiotic

*S. coelicolor* M145 containing pSE34 and the SCO6569–overexpressing *S. coelicolor* M145 strains were inoculated into 50ml of R2YE liquid media containing 50g/ml thistrepton, followed by the growth at 30°C for 5 days. Mycelia and supernatant
were separated by centrifugation at 15,000rpm for 5min. Culture media were extracted and analyzed for quantitative measurement of antibiotics using a UV–visible Spectrophotometer (Shimazu, Japan) as described previously [3, 12, 24].

2.5 Doxorubicin titer measurements

The recursively mutated doxorubicin–overproducing *S. peucetius* industrial mutant strain (generously provided by the Boryung Pharmaceutical Company, Korea) and the wild-type *S. peucetius* strain (*S. peucetius subsp. caesius* ATCC27952, purchased from the American Type Cell Collection) were grown in shake flask cultures in NDYE media (4). Both the supernatant and cell pellet samples were harvested during a 10–day culture period, followed by HPLC analysis for doxorubicin titers and densitometry measurements of cell growth (9, 13).

2.6 Reverse transcriptase polymerase chain reaction (RT-PCR)

Mycelia were harvested and immediately frozen in liquid nitrogen and stored at −80° for RNA extraction. Whole cell RNA was extracted by the RNeasy Mini Kit (QIAGEN GmbH, Germany) method.
DNase I-treated RNA (7ug) was used as a template for reverse transcription (RT) at 50? with the AVM Reverse Transcriptase XL (TaKaRa, Japan) and random hexamers. The resulting cDNA was used for PCR amplification under the following conditions: denaturation at 95? for 30 sec, annealing at 55? for 30 sec, and extension at 68? for 35 sec by 25 cycles. Each primer pair was carefully designed to generate PCR product of approximately 150 to 250 bp. RT-PCR primer sequences pairs (5′–3′) were as follows: rDNA (GACTCCTACGGGAGGCAGCA, CGCCCAATAATTCCGGACAA), actII–ORF4 (TCCCTGGTAAATTCGATCC, CCATGTGCATACGCTGGATT), redZ (ACGTCGGTCGAAGAACTGGT, GAGGAGGACTTCCGTTTCCC) and redD (CCCTGGAGATCTCAGC, GTACGACTCCAGGGCGCTTC) [20–22]. RNA samples that had not submitted to RT reaction were used for PCR as negative controls.
3. Results & Discussions

3.1 Over-expression of proteomics-driven potential target genes in *Streptomyces* species

It was previously reported that the comparative proteomics analyses of two *S. lividans* strains, the wild-type TK21 and *afsR2*-overexpressing ESK206, revealed approximately 340 protein spots showing intensity differences more than 2-fold [13, 14]. In addition, the most noticeable 16 protein spots showing a difference in expression more than 10-fold were characterized using MALDI-TOF [14]. The biological significance of the proteomics-guided targets was verified by selecting the sixteen putative AfsR2 targets including four putative negatives (SCO6569, SCO1860, SCO1947 and SCO5249) as well as twelve putative positives (SCO0512, SCO5737, SCO5262, SCO7800, SCO3649, SCO4228, SCO3834, SCO5520, SCO3548, SCO0267, SCO5254, and SCO4352) [Table 1]. Each of the sixteen genes was PCR-amplified, and cloned into the *Streptomyces* expression vector pSE34 [Fig. 3]. The genes were finally introduced into both *S. lividans* TK21 and *S. coelicolor* M145 via polyethylene glycol (PEG)-mediated protoplast transformation. The level of sporulation and actinorhodin production was visualized by culturing each of the *S. lividans* and *S. coelicolor* transformants containing the plasmid on a R2YE plate containing 50g/ml thioestrepton at 30C for 6 days [Fig. 4].
Table 1. Putative afsR2-dependent genes identified from comparative proteomics.

<table>
<thead>
<tr>
<th>Proteomics-driven target genes</th>
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<tbody>
<tr>
<td>No.</td>
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<tr>
<td>NEG-LP1</td>
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<td>NEG-LP2</td>
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<td>NEG-LP3</td>
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<td>POS-LP11</td>
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<tr>
<td>POS-LP12</td>
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</table>
Figure 3. *Streptomyces* expression plasmid maps of the seventeen *afsR2* target genes.
Figure 4. Morphological phenotypes of transformants grown on R2YE agar plates A) *S. lividans* TK21 transformants containing the *afsR2* target genes, B) *S. coelicolor* M145 transformants containing *afsR2* target genes, C) SCO numbers of *afsR2* target genes, D) the re-streaked *S. coelicolor* transformants harboring the empty expression vector pSE34 alone (right) or overexpressing SCO6569 (left), E) Five-day-old R2YE liquid culture with *S. coelicolor* transformants harboring the empty pSE34 alone (right) or overexpressing SCO6569 (left).
As shown in Fig. 4A, *S. lividans* transformants containing the SCO0512, SCO5737, SCO1860, SCO4228, SCO1947 and SCO0267 genes showed no significant phenotypic difference compared with the *S. lividans* containing the pSE34 vector plasmid. However, *S. lividans* transformants containing the SCO6569, SCO5737, SCO5262, SCO7800, SCO3649, SCO3834, SCO5520, SCO3548, SCO5254, SCO4352, SCO4677, and SCO5249 showed phenotypes of the delayed sporulation. Especially, the *S. lividans* containing SCO3548 gene failed to sporulate even after prolonged incubation, even though no significant reduction of actinorhodin production in *S. coelicolor*. Among *S. coelicolor* transformants tested, only two *S. coelicolor* transformants containing SCO6569 and SCO5262 failed to show normal sporulation as well as actinorhodin production [Fig. 4B]. Since the SCO6569 gene was the only one identified as a putative afsR2-dependent negative target and also showed the repressor phenotype in both *S. lividans* and *S. coelicolor*, the SCO6569 was selected as a possible afsR2-dependent negative regulator [Fig. 4D]. Overexpression of SCO6569 also inhibited biosynthesis of two major *S. coelicolor* antibiotics (more dramatic decrease in actinorhodin and much less in undecylprodigiosin in the liquid cultures) [Fig. 5]. Moreover, transcripts encoded by the pathway-specific activators (*actII-ORF4* for actinorhodin and *redD/Z* for undecylprodigiosin) were similarly reduced in SCO6569-overexpressing *S. coelicolor* [Fig. 6], suggesting that the SCO6569 could be a key AfsR2-dependent down-regulator acting broadly in antibiotic biosynthesis (probably more significant effect on actinorhodinin) in *S. coelicolor*. 
Figure 5. Cultures media were analyzed spectrophotometrically with the absorbance peaks for undecylprodigiosin (und) and actinorhodin (act) at 530 nm and 640 nm, respectively.
In *S. coelicolor* genome, SCO6569 is apparently organized as an operon with two other upstream genes such as SCO6568 (ATP-binding cassette (ABC) transporter integral membrane protein) and SCO6567 (ABC transporter ATP binding protein), suggesting that SCO6569 may be a substrate-binding protein involved in a simple sugar ABC transport system (http://streptomyces.org.uk). Since the AfsR2–induced actinorhodin overproduction in both *S. coelicolor* and *S. lividans* was experimentally verified to be regulated via glycerol-inducible and glucose-repressible mechanism [15], multiple copies of SCO6569 gene might stimulate a glucose-like simple sugar transport into the cell leading to the repression of actinorhodin biosynthesis. Although a similar substrate-binding protein encoded by dasA involved in ABC transport system in *S. griseus* was previously reported and functionally characterized [26], a detailed mechanism of SCO6569 regulation remains to be further characterized. In addition, *S. coelicolor* transformant containing SCO5262, which was identified as an afsR2-dependent positive target on the basis of the results from comparative proteomics analyses [14], however, failed to produce actinorhodin as shown in the present study [Fig. 4B]. Further study will be needed to determine whether this phenotypic change is related to the AfsR2 function.
Figure 6. Gene expression analysis of the *actII*-ORF4, *redD*, and *redZ* genes by RT-PCR with the RNA samples of five-day-old R2YE liquid cultures. Lane M, 100bp size marker; lane 1-2, rDNA, lane 3-4, *actII*-ORF4 lane 5-6, SCO6569 lane 7-8, *redD* lane 9-10, *redZ* Odd number lanes, RT-PCR with total RNA from *S. coelicolor* transformants harboring the vector pSE34; even number lanes, RT-PCR with total RNA from *S. coelicolor* transformants overexpressing SCO6569. Only the DNA fragment containing RBS and ORF of SCO6569 without its own promoter was cloned under the *ermE* promoter, leading to the increased expression of *ermE*-driven SCO6569 in a high-copy pSE34 plasmid. Each primer pair (20 mer) was designed to generate PCR product of approximately 150 to 250 bp.
In conclusion, the comparative proteomics followed by the functional expression describe here can be an efficient approach to identify previously-unknown $afsR2$-dependent target genes. Potentially, further manipulation of this negative regulator such as SCO6569 may result in further improvements in the productivity of pharmaceuticals produced by industrial *Streptomyces* strains, including those for which complete genome sequence information and knowledge of regulatory mechanisms at the molecular level are not currently available.
3.2 Over-expression of transcriptomics-driven potential target genes in *Streptomyces* species.

To detect global changes in mRNA abundance associated with overproduction of doxorubicin in *S. peucetius*, comparative transcriptome analyses in cultures of the wild-type and mutant strains of *S. peucetius* were conducted using *S. coelicular* cDNA microarrays containing targets of known sequence. Initially, approximately 160 *S. coelicolor* potential candidate genes showing at least a two-fold change in transcription between the wild-type and mutant strain in at least one time point were identified.
**Table 2.** Putative regulatory genes identified from cDNA microarray

<table>
<thead>
<tr>
<th>No.</th>
<th>Identification</th>
<th>SCO number</th>
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<tbody>
<tr>
<td>POS1</td>
<td>putative ECF-subfamily sigma factor</td>
<td>5147</td>
</tr>
<tr>
<td>POS2</td>
<td>putative polysaccharide synthesis gene</td>
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After further analyses of the growth-phase dependent transcription profiles of these potential candidate genes, 20 genes showing particularly large transcriptional changes between two strains were selected (Fig. 2B, C) and individually over-expressed in *S. coelicolor* under control of the strong ermE*-promoter of the pES34 *Streptomyces* expression vector. Among these genes, the most prominent increase in production of the blue pigment antibiotic actinorhodin was induced by SCO5147 as a putative positive regulator. The greatest decrease in actinorhodin expression was observed in cells expressing SCO3579 (Fig. 3A). Expression of the *S. peucetius* homolog of SCO5147 in the *S. peucetius* industrial mutant strain was 4 fold higher than in the *S. peucetius* wild-type strain at the T3 time point (Fig. 2B), whereas the *S. peucetius* homolog of SCO3579 was repressed in the industrial mutant strain by 50 % (Fig. 2C). Real-time RT-PCR targeting these two potential regulatory genes in the *S. peucetius* strains confirmed the transcriptional perturbations observed by microarray analysis (data not shown).
Figure 7. Schematic map of over-expression constructs
Actinorhodin production of *S. coelicolor* M145 constructs

**Figure 8.** Actinorhodin production by various target genes containing plasmid into *S. coelicolor* M145
The putative positive regulatory gene, SCO5147 previously has been classified as one of the 50 ECF-subfamily sigma factor genes present in the genome of *S. coelicolor*. Its biological role has not been known (http://streptomyces.org.uk/), although our inability to delete it in *S. coelicolor* suggests that its function is essential to bacterial viability (Huang et al., unpublished data). The putative negative regulatory gene, SCO3579 was previously proposed as a *whiB*-like putative transcription factor gene named *wblA* in *S. coelicolor* (http://streptomyces.org.uk/, 16). Although *whiB* is developmental regulatory gene identified and characterized in *S. coelicolor* as being essential for sporulation of aerial hyphae, the biological function of *wblA* with regard to secondary metabolite regulation previously has not been known (16). To examine directly the biological effects of these two potential candidate genes on doxorubicin overproduction, each of the *S. coelicolor* SCO5147 and *wblA*, SCO1712 genes cloned in the pES34 was introduced into the *S. peucetius* mutant strain by transformation. While constitutive expression of the SCO5147 gene in the mutant was associated in some experiments with a further increase in doxorubicin production in liquid cultures, this positive effect was not consistently observed in liquid cultures and was not detected at all in plate cultures (Fig. 9).
**Effect of selected target genes during cell growth**

*Figure 9.* Actinorhodin production and sporulation by SCO3579 (red star) and SCO5147 (blue star) genes containing plasmid into *S. coelicolor* M145.
**Figure 10.** Actinorhodin production and sporulation by SCO1712 (green star) gene containing plasmid into *S. coelicolor* M145.
Figure 11. Absorbance spectra of pigments produced by *S. coelicolor* M145 containing pSE34 and *S. coelicolor* M145 containing SCO3579 (red star) and SCO1712 (green star) strains into R2YE liquid culture supernatant. und, undecylprodigiosin; act, actinorhodin.
Additionally, introduction of a multicopy plasmid carrying the positive regulatory gene SCO5147 into the wild type *S. peucetius* strain (ATCC27952) did not produce a detectable increase in doxorubicin production (data not shown), arguing that SCO5147 overexpression per se is insufficient for doxorubicin overproduction. In contrast, introduction of the *wblA* and SCO1712 genes in the *S. peucetius* doxorubicin–overproducing mutant resulted in a dramatically reduced production of the red doxorubicin pigment during growth in liquid media as well as in plate cultures (Fig. 15). The 420-bp putative promoter–containing regions 5’ to the predicted SCO5147 ORF and predicted *wblA* ORF, predicted SCO1712 ORF were cloned and sequenced for the *S. peucetius* wild-type strain and the industrial mutant strain. No sequence difference was observed in these regions between the wild type and industrial strain (data not shown), that the differential expression of SCO5147 and *wblA*, SCO1712 in these strains is a consequence of mutations in regulatory proteins rather than in the promoter.
Figure 12. Doxorubicin production by SCO5147 and SCO3579 genes containing plasmid into S. peucetius BRdox.
Quantitative analysis of DXR productivity

*Figure 13.* Volumetric doxorubicin productivity by pSE34-containing (white star), SCO5147-containing (blue star) and SCO3579-containing (red star) *S. peucetius* BRdox.
Figure 14. Doxorubicin production by SCO1712 (green star) gene containing plasmid into S. peucetius BRdox.
Figure 15. Volumetric doxorubicin productivity by pSE34–containing (white star) and SCO1712–containing (green star) *S. peucetius* BRdox.
Overexpression of \( wblA \) also inhibited the biosynthesis of actinorhodin (Act) in \( S. coelicolor \) (Fig. 3A, B) as well as the synthesis of two other \( S. coelicolor \) antibiotics: undecylprodigiosin (Red) and calcium-dependent antibiotic (CDA) in \( S. coelicolor \). Moreover, transcripts encoded by activators of biosynthesis of each of the three major \( S. coelicolor \) antibiotics (i.e., \( actII-ORF4 \) for actinorhodin, \( redD/Z \) for undecylprodigiosin, and \( cdaR \) for CDA) were reduced in \( wblA \)-overexpressing \( S. coelicolor \) and \( SCO1712 \)-overexpressing \( S. coelicolor \) (Fig. 3C), suggesting that \( wblA \) and \( SCO1712 \) act broadly to down-regulate antibiotic biosynthesis in this organism. A search for conserved motifs within regions 5’ to ORFs of the \( actII-ORF4, redD/Z \) and \( cdaR \) genes revealed no commonality except for 5’ UTR sequences known to represent ribosome binding sites (AGGAG) (data not shown), ---suggesting that WblA, whose sequence suggests that it may be a DNA binding protein, does not act directly as a transcriptional repressor of these genes. Aerial mycelia formation was also decreased in the \( S. coelicolor \) transformant, which consequently showed a bald phenotype (Fig. 3A), implying that \( wblA \) modulates morphological differentiation as well as antibiotic biosynthesis in \( Streptomyces \).
**Gene expression analysis of antibiotic pathway specific activators by RT-PCR**

**Figure 16.** Gene expression analysis of the actII–ORF4, redD, redZ, and cdaR genes by RT-PCR. Lane M, 100bp size marker; lane 1–2, actII–ORF4 lane 3–4, redD lane 5–6, redZ lane 7–8, cdaR; lane 9–10, wblA lane 11–12, rDNA; Odd number lanes, RT-PCR with total RNA from *S. coelicolor* transformants harboring the vector pSE34; even number lanes, RT-PCR with total RNA from *S. coelicolor* transformants overexpressing *wblA*. Only the DNA fragment containing RBS and ORF of *wblA* without its own promoter was cloned under the ermE* promoter, leading to the increased expression of ermE*-driven *wblA* in a high-copy pSE34 plasmid. Each primer pair (20 mer) was designed to generate PCR product of approximately 150 to 250 bp. RT-PCR primer sequences pairs (5’–3’) were as follows: rDNA, actII–ORF4, redD, redZ and cdaR.
**Figure 17.** Gene expression analysis of the *actII-ORF4, redD, redZ,* and *cdar* genes by RT-PCR. Lane M, 100bp size marker; lane 1–2, *actII-ORF4* lane 3–4, *redD* lane 5–6, *redZ* lane 7–8, *cdar*; lane 9–10, SCO1712 lane 11–12, rDNA; Odd number lanes, RT-PCR with total RNA from *S. coelicolor* transformants harboring the vector pSE34; even number lanes, RT-PCR with total RNA from *S. coelicolor* transformants overexpressing SCO1712. Only the DNA fragment containing RBS and ORF of SCO1712 without its own promoter was cloned under the ermE* promoter, leading to the increased expression of ermE*-driven SCO1712 in a high-copy pSE34 plasmid. Each primer pair (20 mer) was designed to generate PCR product of approximately 150 to 250 bp. RT-PCR primer sequences pairs (5’–3’) were as follows: rDNA, *actII-ORF4, redD, redZ* and *cdar*.
References


