Transcriptome Analysis of an Antibiotic Downregulator Mutant and Synergistic Actinorhodin Stimulation via Disruption of a Precursor Flux Regulator in *Streptomyces coelicolor*^{▽†}

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Through microarray analysis of an antibiotic-downregulator-deleted *Streptomyces coelicolor* $\Delta wblA$ Δ SCO1712 mutant, 28 *wblA*- and SCO1712-dependent genes were identified and characterized. Among 14 *wblA*- and SCO1712-independent genes, a carbon flux regulating 6-phosphofructokinase SCO5426 was additionally disrupted in the $\Delta wblA$ Δ SCO1712 mutant and further stimulated actinorhodin production in *S. coelicolor*, implying that both regulatory and precursor flux pathways could be synergistically optimized for antibiotic production.

Members of the bacterial genus *Streptomyces* produce the majority of known microbial-origin antibiotics as well as a number of other secondary metabolites, including many pharmaceutically valuable compounds (6, 11). It has been proposed that the expression of antibiotic-biosynthetic genes is tightly controlled through multiple different regulatory networks in *Streptomyces* species (18, 19). Traditionally, random mutation has been one of the most widely used strategies for *Streptomyces* strain improvement with regard to the generation of secondary-metabolite-overproducing industrial mutants, even though the molecular genetic basis underlying such enhanced production remains largely unknown (17, 20, 21, 23). Recent “-omics”-guided reverse engineering approaches, including comparative transcriptomics and proteomics were successfully used to identify alterations in gene expression associated with the overproduction of secondary metabolites in industrial streptomycete strains (14, 17).

Streptomyces coelicolor has been widely used as a model organism for molecular genetic studies of secondary metabolism, regulation of antibiotic biosynthesis, and morphological differentiation in *Streptomyces* species (2, 7, 12). In general, secondary metabolism in *S. coelicolor* is regulated by pathway-specific regulatory proteins belonging to the SARPs (*Streptomyces* antibiotic-regulatory proteins), such as actinorhodin (ACT)-specific ActII-open reading frame 4 (ORF4), undecylprodigiosin (RED)-specific RedD, and calcium-dependent antibiotic (CDA)-specific CdaR (1, 3, 24) as well as many pleiotropic regulators, including the recently identified WblA (9, 13, 14). WblA, which is a novel downregulator that was identified by comparing gene transcription profiles using DNA microarrays, was shown to inhibit the biosynthesis of doxorubicin (DXR) in *Streptomyces peucetius* as well as the production of the three above-mentioned antibiotics, ACT, RED, and CDA,

in *S. coelicolor*, suggesting that WblA acts globally in *Streptomyces* species as a downregulator of antibiotic biosynthesis (14). In addition, a *tetR* family transcriptional regulatory gene, SCO1712, was identified to be a *wblA*-independent antibiotic downregulator because its disruption in *S. coelicolor* not only upregulated antibiotic biosynthesis through pathway-specific regulators in the presence of the *wblA* transcript but also further stimulated antibiotic production in the *wblA* deletion mutant (16).

These previous results suggest that comparative transcriptome analysis using *S. coelicolor* microarrays with antibiotic-overproducing mutants may be an effective approach to discover novel regulatory genes and mechanisms in *Streptomyces* species. Moreover, sequential targeted gene disruptions of independently working regulatory systems could provide an efficient and rational alternative approach for *Streptomyces* strain improvement (14, 16). Here, *wblA*- and SCO1712-dependent and *wblA*- and SCO1712-independent genes were identified using an additional comparative microarray analysis of an antibiotic-downregulator-deleted *S. coelicolor* $\Delta wblA$ Δ SCO1712 mutant, followed by analysis of their functional expression and by reverse transcription-PCR (RT-PCR). Moreover, among the *wblA*- and SCO1712-independent genes identified, SCO5426, an ACT precursor flux-downregulating 6-phosphofructokinase gene, was additionally disrupted in the *S. coelicolor* $\Delta wblA$ Δ SCO1712 mutant background, and this disruption further stimulated ACT production, implying that both ACT biosynthesis-regulatory and precursor flux-controlling pathways could be synergistically optimized for antibiotic production in *Streptomyces* species.

A comparative transcriptome analysis was conducted using an *S. coelicolor* whole-genome chip containing the *S. coelicolor* wild type and an *S. coelicolor* $\Delta wblA$ Δ SCO1712 double-knock-out mutant (16). Each strain was grown in a 5-liter bioreactor (Biotron, South Korea) containing 2 liters of modified R5 medium (15). Total RNAs were isolated during the early exponential stage at 11 h, 19 h, 30 h, and 40 h. An *S. coelicolor* genomic DNA microarray chip containing 8,000 oligonucleotides was commercially manufactured based on the pub-

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FIG. 1. (A) Microarray analysis of the gene expression pattern of the ACT biosynthetic gene cluster on the *S. coelicolor* Δ wblA Δ SCO1712 mutant compared with the *S. coelicolor* wild type. RNA samples were isolated at 11 h, 19 h, 30 h, and 40 h during growth on modified R5 medium. The red color indicates higher transcript abundance and the green color indicates lower transcript abundance in the *S. coelicolor* Δ wblA Δ SCO1712 mutant strain than in wild-type *S. coelicolor*. The black color represents equal transcript abundances in the two strains. CoA, coenzyme A. (B) Real-time RT-PCR analysis of the *actII-ORF4* transcript in the *S. coelicolor* M145 wild type, Δ wblA mutant, Δ wblA Δ SCO1712 mutant, and Δ wblA Δ SCO1712 Δ SCO5426 mutant. The y-axis scale represents the expression value relative to that of *hrdB*, a housekeeping sigma factor, which was set to 1. (C) *wblA*- and SCO1712-dependent genes (top microarray) that were upregulated and downregulated (lower microarray) by more than 5-fold at two different time points. (D) *wblA*- and SCO1712-independent genes, including SCO5426 (red star), showing no significant changes between the two strains.

licly available complete genome sequence information (<http://streptomyces.org.uk>). Each probe length was 35- to 40-mers, and biotinylated RNAs were prepared from 0.5 μ g total RNAs using the CombiMatrix TotalPrep RNA amplification kit (Ambion; MessageAmp II-Biotin Enhanced, MessageAmp II-Bacteria). Following fragmentation, 1- to 5- μ g RNA fragments were hybridized to the CombiMatrix microarray according to the protocols provided by the manufacturer. The arrays were scanned using the high-resolution Axon Instruments Genepix 4000B and 4200A microarray scanners (MacroGen Inc., South Korea).

From the comparative transcriptome analysis of the *S. coelicolor* wild type and an *S. coelicolor* Δ wblA Δ SCO1712 double-knockout mutant, 875 genes were found to have a 2-fold or greater change in expression at two or more time points, many of which were assigned as hypothetical proteins, regulatory proteins, membrane proteins, secreted proteins, and biosyn-

thetic cluster gene products (see Fig. S1 in the supplemental material). Not surprisingly, many genes associated with the biosyntheses of Act, Red, and CDA in *S. coelicolor* were up-regulated in an *S. coelicolor* Δ wblA Δ SCO1712 double-knockout mutant relative to levels of regulation in the wild type (Fig. 1A and see Fig. 2SA in the supplemental material). Especially, expression of Act cluster pathway genes in the *S. coelicolor* Δ wblA Δ SCO1712 mutant was 11.67-fold higher on average than those of the wild type at the 30-h time point. During these time periods, expression of the ACT pathway-specific regulatory gene, *actII-ORF4*, was enhanced not only in the Δ wblA Δ SCO1712 double-knockout mutant but also in each single-knockout mutant, the Δ wblA or Δ SCO1712 strain, further confirming that *wblA* and SCO1712 can independently function as antibiotic-downregulatory genes by controlling a pathway-specific regulatory gene (Fig. 1B).

After further analyses of the growth-phase-dependent transcription profiles of *wblA*- and SCO1712-dependent potential-candidate genes, a total of 28 (13 upregulatory and 15 downregulatory) genes showing a >5-fold change in transcription at more than 2 time points between the two strains were selected (Fig. 1C). To determine which gene(s) among these 28 *wblA*- and SCO1712-dependent targets is regulated by *wblA*, SCO1712, or both, total RNA samples were prepared from three mutants, *S. coelicolor* $\Delta wblA$, *S. coelicolor* $\Delta SCO1712$, and *S. coelicolor* $\Delta wblA \Delta SCO1712$ 30 h after the start of growth and used as templates for gene expression analysis by RT-PCR (see Fig. S3 in the supplemental material). Primers for RT-PCR were specific to sequences within the 28 *wblA*- and SCO1712-dependent target genes (see Table S1 in the supplemental material) and were designed to produce cDNAs of approximately 200 bp. A primer pair designed to amplify a cDNA from a housekeeping sigma factor gene, *hrdB*, was used as an internal control, and the RT-PCR analysis was carried out at least two times for each primer pair. As shown in Fig. 2A, the RT-PCR analyses demonstrated that transcripts encoded by SCO0268 (encoding a hypothetical protein), SCO0297 (encoding a secreted protein), and SCO6828 (encoding a secreted protein) were increased relative to transcription levels in the $\Delta wblA$ mutant, while transcripts of SCO5073 (encoding oxidoreductase) and SCO6100 (encoding phosphoadenosine phosphosulfate reductase) were increased in both the $\Delta wblA$ and $\Delta SCO1712$ mutants. Meanwhile, expression levels of the $\Delta wblA$ - and $\Delta SCO1712$ -dependent downregulatory genes, including SCO0682 (encoding a hypothetical protein), SCO0685 (encoding a hypothetical protein), SCO4173 (encoding a hypothetical protein), SCO4174 (encoding an integral membrane protein), and SCO5351 (encoding a regulatory protein) seemed to be downregulated only in the $\Delta wblA$ mutant (Fig. 2A). All the genes tested by RT-PCR analyses shown in Fig. 2A were also confirmed by real-time RT-PCR (see Fig. S4 in the supplemental material). Unfortunately, no conserved or common motif located in the upstream regions of these genes was identified. Although it is still not clear how these *wblA*- and SCO1712-dependent genes are interconnected, these downstream genes are believed to be indirectly regulated by *wblA*, SCO1712, or both. Taken together, these results strongly suggest that some downstream target genes of *wblA* and SCO1712 are somehow coregulated, while the others are controlled by either *wblA* or SCO1712 separately.

To verify the biological significance of the potential *wblA*- and SCO1712-dependent targets, these 28 genes were individually cloned under the strong and constitutive promoters of the *Streptomyces* integrative expression vector *ermEpSET152* (Fig. 2B), followed by intergeneric conjugation into wild-type *S. coelicolor* (4, 8). Primers for cloning are shown in Table S2 in the supplemental material, and all the overexpression strains were verified by genomic DNA PCR analysis (data not shown). Among these genes, the most prominent increase in production of the blue-pigment antibiotic ACT from the cells grown in a modified R5 agar plate for 5 days was induced by SCO0297 (encoding a secreted protein). The greatest decrease in ACT production under the same conditions was observed in cells expressing SCO2964 (encoding a LysR family transcriptional regulator) (Fig. 2D). To quantitatively measure ACT productivity, each strain was cultured in 50 ml modified R5 medium

for 6 days using a baffled flask and the control strain containing the inserted empty vector (15). As expected, a positive SCO0297-overexpressing and a negative SCO2964-overexpressing *S. coelicolor* strain produced ACT approximately 3-fold more and 2-fold less than the wild type, respectively (see Fig. S2B and S2C in the supplemental material). As shown in Fig. 2A (see also Fig. S4A in the supplemental material), the expression of SCO0297 was positively regulated by *wblA* alone, but the *wblA* and/or SCO1712 dependency of the negative SCO2964 transcript was not clear due to an intrinsically low expression level even in the wild-type *S. coelicolor* strain as determined via real-time RT-PCR (see Fig. S5B in the supplemental material). Potential additional stimulation of ACT productivity by either the addition of positive SCO0297 or deletion of negative SCO2964 was assessed in an *S. coelicolor* $\Delta wblA \Delta SCO1712$ mutant background. However, no significant additional ACT stimulation was observed in either strain (data not shown), suggesting that additional modification of these *wblA*- and SCO1712-dependent downstream genes, such as SCO0297 and SCO2964, might not influence any further antibiotic overproduction because they were already maximally regulated due to the deletion of upstream regulatory genes, such as *wblA* and SCO1712, in the *S. coelicolor* double-knockout mutant.

Further comparative transcriptome analysis between the *S. coelicolor* wild type and an *S. coelicolor* $\Delta wblA \Delta SCO1712$ double-knockout mutant revealed an additional 14 genes that displayed no particular transcriptional changes (a <1.2-fold change) during the same sampling period (Fig. 1D). These putative *wblA*- and SCO1712-independent genes included a carbon flux-regulating gene, SCO5426, which is one of the three 6-phosphofructokinase genes. Recently, SCO5426 disruption was reported to enhance precursor carbon flux as well as NADPH supply for ACT biosynthesis through the activation of the pentose phosphate pathway, resulting in significantly enhanced ACT production in *S. coelicolor* (5). Based on the fact that SCO5426 expression in the *S. coelicolor* $\Delta wblA \Delta SCO1712$ double-knockout mutant exhibited no significant change in the comparative transcriptome analysis, additional deletion of this *wblA*- and SCO1712-independent ACT precursor flux-downregulating SCO5426 gene in the absence of both *wblA* and SCO1712 might further enhance ACT productivity in *S. coelicolor*. To test this hypothesis, we also disrupted SCO5426 in the *S. coelicolor* $\Delta wblA \Delta SCO1712$ double-knockout strain via a PCR-targeted gene disruption system (10). SCO5426 carried by the St6A11 cosmid was replaced with a spectinomycin resistance/*oriT* cassette, generating pSt6A11 Δ 5426 (<http://streptomyces.org.uk/>), which was introduced into *S. coelicolor* M145 by conjugative gene transfer (Fig. 3A). Construction of the triple-knockout mutant by additional disruption of SCO5426 in the *S. coelicolor* $\Delta wblA \Delta SCO1712$ background was confirmed by PCR analysis. The expected 1.2-kb size for the PCR-amplified bands was observed in genomic DNA samples isolated from *S. coelicolor* $\Delta wblA \Delta SCO1712$, while a band of the expected size (1.7 kb) was observed in genomic DNA samples isolated from the *S. coelicolor* $\Delta wblA \Delta SCO1712 \Delta SCO5426$ mutant (Fig. 3A), implying that SCO5426 was specifically and additionally disrupted, as expected, in the *S. coelicolor* $\Delta wblA \Delta SCO1712$ double-knockout mutant.

FIG. 2. Real-time RT-PCR analysis of various target genes. Real-time RT-PCR was performed in a Thermal Cycler Dice real-time system (TaKaRa, Japan). The primers were mixed with cDNA as well as SYBR premix Ex Taq. The y-axis scale represents expression values relative to that of *hrdB*, a housekeeping sigma factor, whose value was set as 1. (A) Putative *wblA*- and SCO1712-dependent upregulators: SCO0268 (hypothetical protein), SCO0297 (secreted protein), SCO5073 (oxidoreductase), SCO6100 (phosphoadenosine phosphosulfate reductase), and SCO6828 (secreted protein). (B) Putative *wblA*- and SCO1712-dependent downregulators: SCO0682 (hypothetical protein), SCO0685 (hypothetical protein), SCO4173 (hypothetical protein), SCO4174 (integral membrane protein), and SCO5351 (regulatory protein). (C) Plasmid map of the *Streptomyces* integrative expression vector containing the *ermE*^{*} promoter (4, 15). The *wblA*- and SCO1712-dependent target genes were subcloned into this plasmid, and each recombinant plasmid was introduced into the *S. coelicolor* M145 wild type by interspecies conjugation from *Escherichia coli* ET12567/pUZ8002. RBS, ribosome binding site. (D) Five-day-old modified R5 agar plate cultures of *S. coelicolor* M145 wild type by interspecies conjugation from *Escherichia coli* ET12567/pUZ8002. The plates show the overexpression construct of *wblA*- and SCO1712-dependent target genes along with the one containing the empty expression vector pSET152 alone.

The *S. coelicolor* Δ *wblA* Δ SCO1712 Δ SCO5426 triple-knockout mutant along with the previously generated single- and double-knockout mutants was cultured on modified R5 plates as well as liquid media for ACT production. The plates were then visually observed. There seemed to be no significant phenotypic difference observed among single-, double-, and triple-

knockout mutants (Fig. 3B). While all mutant strains exhibited comparable growth patterns in 8-day liquid fermentation cultures (Fig. 3C), the *S. coelicolor* Δ *wblA* Δ SCO1712 Δ SCO5426 triple-knockout mutant strain exhibited the highest ACT-specific productivity (0.18 g/g [dry weight] cells [DCW] at 168 h), which was 1.7-fold and 1.3-fold higher than those of the single-

FIG. 3. (A) Schematic representation of PCR-targeted gene disruption of SCO5426. SCO5426 was changed to a spectinomycin resistance gene (*spe*) and *oriT* fragment. Gene replacement was confirmed by PCR. The PCR products in lanes 1 to 3 were amplified using primer 1-primer 2 pairs, and the PCR products in lanes 6 to 8 were amplified using primer 1-primer 3 pairs. Lanes: 1 and 6, *S. coelicolor* $\Delta wblA \Delta SCO1712$ genomic DNA; 2, 3, 7, and 8, *S. coelicolor* $\Delta wblA \Delta SCO1712 \Delta SCO5426$ genomic DNAs from two independent exconjugant colonies. (B) Five-day-old modified R5 agar plate cultures of the *S. coelicolor* M145 wild type (right) and the $\Delta wblA$ (bottom), $\Delta wblA \Delta SCO1712$ (left), and $\Delta wblA \Delta SCO1712 \Delta SCO5426$ (top) mutants for 5 days. Time-dependent growth curve (C) and ACT-specific productivities (D) of wild-type *S. coelicolor* M145, *S. coelicolor* $\Delta wblA$, *S. coelicolor* $\Delta wblA \Delta SCO1712$, and *S. coelicolor* $\Delta wblA \Delta SCO1712 \Delta SCO5426$ cultured in modified R5 medium for 8 days in a 2-liter bioreactor (22). The averages of two independent fermentation results are shown with error bars.

knockout *S. coelicolor* $\Delta wblA$ (0.10 g/g DCW at 168 h) and the double-knockout *S. coelicolor* $\Delta wblA \Delta SCO1712$ (0.14 g/g DCW at 168 h) mutant strains, respectively (Fig. 3D), suggesting that the additional deletion of a *wblA*- and SCO1712-independent ACT precursor flux-downregulating SCO5426 gene led to further stimulation of ACT biosynthesis. As shown in Fig. 1B, *actII-ORF4* expression in the triple-knockout mutant was consistently higher than that in the others during the early culture period. Although similar *actII-ORF4* stimulation was also reported in a previously generated SCO5426 single-knockout mutant strain (5), it remains to be understood how the additional deletion of ACT precursor flux-downregulating SCO5426 is related to the enhanced transcription of the ACT pathway-specific regulatory *actII-ORF4* region in the $\Delta wblA \Delta SCO1712$ double-knockout strain.

In conclusion, some downstream regulatory genes under the control of *wblA* and SCO1712 are connected and coregulated,

while others are controlled by either *wblA* or SCO1712 separately. The *wblA*- and SCO1712-dependent downstream genes might not be further manipulated to stimulate ACT biosynthesis due to complete deletion of both *wblA* and SCO1712 in the *S. coelicolor* double-knockout mutant. Sequential targeted gene disruption of independently working downregulators as well as precursor flux downregulators could synergistically provide an efficient and rational strategy for *Streptomyces* strain improvement.

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REFERENCES

1. Arias, P., M. A. Fernandez-Moreno, and F. Malpartida. 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.
2. Bibb, M. J. 1996. The regulation of antibiotic production in *Streptomyces coelicolor* A3(2). *Microbiology* **142**:1335–1344.
3. Bibb, M. J. 2005. Regulation of secondary metabolism in streptomycetes. *Curr. Opin. Microbiol.* **8**:208–215.
4. Bibb, M. J., G. R. Janssen, and J. M. Ward. 1985. Cloning and analysis of the promoter region of the erythromycin-resistance gene (*ermE*) of *Streptomyces erythraeus*. *Gene* **38**:215–216.
5. Borodina, I., et al. 2008. Antibiotic overproduction in *Streptomyces coelicolor* A3(2) mediated by phosphofructokinase deletion. *J. Biol. Chem.* **283**:25186–25199.
6. Challis, G. L., and D. A. Hopwood. 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc. Natl. Acad. Sci. U. S. A.* **100**:14555–14561.
7. Chater, K. F., et al. 2002. *Streptomyces coelicolor* A3(2): from genome sequence to function. *Methods Microbiol.* **33**:321–336.
8. Choi, S. U., C. K. Lee, Y. I. Hwang, H. Kinoshita, and T. Nihira. 2004. Intergenic conjugal transfer of plasmid DNA from *Escherichia coli* to *Kitasatospora setae*, a bafilomycin B1 producer. *Arch. Microbiol.* **181**:294–298.
9. Floriano, B., and M. Bibb. 1996. *afsR* is a pleiotropic but conditionally required regulatory gene for antibiotic production in *Streptomyces coelicolor* A3(2). *Mol. Microbiol.* **21**:385–396.
10. Gust, B., G. L. Challis, K. Fowler, T. Kieser, and K. F. Chater. 2003. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc. Natl. Acad. Sci. U. S. A.* **100**:1541–1546.
11. Hopwood, D. A. 1999. Forty years of genetics with *Streptomyces*: from in vivo through in vitro to in silico. *Microbiology* **145**:2183–2202.
12. Hopwood, D. A., K. F. Chater, and M. J. Bibb. 1995. Genetics of antibiotic production in *Streptomyces coelicolor* A3(2), a model streptomycete. *Biotechnology* **28**:65–102.
13. Horinouchi, S., O. Hara, and T. Beppu. 1983. Cloning of a pleiotropic gene that positively controls biosynthesis of A-factor, actinorhodin, and prodigiosin in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans*. *J. Bacteriol.* **155**:1238–1248.
14. Kang, S. H., et al. 2007. Interspecies DNA microarray analysis identifies WblA as a pleiotropic down-regulator of antibiotic biosynthesis in *Streptomyces*. *J. Bacteriol.* **189**:4315–4319.
15. Kieser, T., M. J. Bibb, K. F. Chater, M. J. Butler, and D. A. Hopwood. 2000. Practical *Streptomyces* genetics: a laboratory manual. John Innes Foundation, Norwich, United Kingdom.
16. Lee, H. N., et al. 2010. Putative TetR family transcriptional regulator SCO1712 encodes an antibiotic downregulator in *Streptomyces coelicolor*. *Appl. Environ. Microbiol.* **76**:3039–3043.
17. Lum, A. M., J. Huang, C. R. Hutchinson, and C. M. Kao. 2004. Reverse engineering of industrial pharmaceutical-producing actinomycete strains using DNA microarrays. *Metab. Eng.* **6**:186–196.
18. Niraula, N. P., S. H. Kim, J. K. Sohng, and E. S. Kim. 2010. Biotechnological doxorubicin production: pathway and regulation engineering of strains for enhanced production. *Appl. Microbiol. Biotechnol.* **87**:1187–1194.
19. Noh, J. H., S. H. Kim, H. N. Lee, S. Y. Lee, and E. S. Kim. 2010. Isolation and genetic manipulation of the antibiotic down-regulatory gene, wblA ortholog for doxorubicin-producing *Streptomyces* strain improvement. *Appl. Microbiol. Biotechnol.* **86**:1145–1153.
20. Park, H. S., et al. 2003. Development of doxorubicin overproducing *Streptomyces* strain using protoplast regeneration. *Sanop Misaengmul Hakhoe Chi* **18**:289–293.
21. Rodriguez, E., et al. 2003. Rapid engineering of polyketide overproduction by gene transfer to industrially optimized strains. *J. Ind. Microbiol. Biotechnol.* **30**:480–488.
22. Ryu, Y. G., M. J. Butler, K. F. Chater, and K. J. Lee. 2006. Engineering of primary carbohydrate metabolism for increased production of actinorhodin in *Streptomyces coelicolor*. *Appl. Environ. Microbiol.* **72**:7132–7139.
23. Vetrivel, K. S., and K. Dharmalingam. 2001. Isolation and characterization of stable mutants of *Streptomyces peucetius* defective in daunorubicin biosynthesis. *J. Genet.* **80**:31–38.
24. White, J., and M. Bibb. 1997. *bldA* dependence of undecylprodigiosin production in *Streptomyces coelicolor* A3(2) involves a pathway-specific regulatory cascade. *J. Bacteriol.* **179**:627–633.