The Journal of Microbiology (2011) Vol. 49, No. 1, pp. 155-160
DOI 10.1007/s12275-011-0330-z

NOTE

Characterization of Sgr3394 Produced only by the A-Factor-Producing *Streptomyces griseus* IFO 13350, not by the A-Factor Deficient Mutant HH1

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(Received August 31, 2010 / Accepted January 14, 2011)

Protein D (9.7 kDa) is an extracellular protein detected in the culture broth of A-factor-producing *Streptomyces griseus* IFO 13350, but not of the A-factor-deficient mutant strain *S. griseus* HH1. Comparison of the N-terminal amino acid sequence with the genomic sequencing data of *S. griseus* IFO 13350 identified protein D as Sgr3394, which encodes a putative secretory protein with unknown function. The premature Sgr3394 consisted of 128 amino acids (13.5 kDa), showed 87.5% identity with SACT1DRAFT-0503, from *Streptomyces* sp. ACT-1, and 68.8% identity with SrosN15-18634, from *S. roseosporus* NRRL15998, and was confirmed to be matured for secretion by a peptide cleavage between the Ala-38 and Ala-39 bond. RT-PCR anaylsis of Sgr3394 clearly showed that it can be transcribed in the wild-type strain, but not in the A-factor-deficient strain. However, a gel-mobility shift assay of the promoter region of *sgr3394* with A-factor-dependent transcriptional regulator (AdpA) showed that AdpA could not specifically recognize the putative AdpA-binding site (5′-TCCCCCGAAT-3′). All of these data strongly suggest that the expression of *sgr3394* is not directly induced by AdpA but is regulated indirectly by an A-factor dependent protein. Introduction of *sgr3394* on a high-copy-numbered plasmid (pWHM3-*sgr3394*) into *S. lividans* TK21 induced massive production of actinorhodin (blue pigment) and undecylprodigiosin (red pigment). Compared to the control, production of each pigment increased by 6.1 and 2.6 times, respectively, on R2YE agar, and 3.1 and 1.4 times, respectively, in R2YE broth; there was little influence on morphogenesis. In *S. coelicolor* A3(2)/pWHM3-*sgr3394*, actinorhodin and undecylprodigiosin productions were enhanced to 1.8 and 1.1 times those observed in the control, respectively, suggesting that overexpression of *sgr3394* can stimulate secondary metabolism, especially actinorhodin biosynthesis, in *S. lividans* and *S. coelicolor*.

***Keywords*:** *Streptomyces griseus*, *adpA*, *sgr3394*, actinorhodin

Streptomyces griseus is well known for producing various kinds of antibiotics, including streptomycin. According to a molecular study on *S. griseus*, a microbial hormone, A-factor (2-isocapryloyl-3*R*-hydroxymethyl-γ-butyrolactone), gradually accumulates in the cell and medium in a growth-dependent manner and acts as a chemical signal molecule that triggers both production of secondary metabolites and formation of spores (Nishida *et al*., 2007). A-factor is released from the cell and binds to the A-factor receptor protein A (ArpA) repressor protein with high affinity, resulting in the dissociation of ArpA from the operator region and thereby leading to active transcription of *adpA*, which acts as a key transcriptional activator in the A-factor regulatory cascade (Yamazaki *et al*., 2004). *S. griseus* is also known to produce many extracellular proteases, including chymotrypsin proteases (SprA, B, and D) (Kim *et al*., 2004), trypsin proteases (SprT and SprU) (Chi *et al*., 2005), and metalloendopeptidase (SgmA) (Kato *et al*., 2002); all of them are positively regulated by AdpA at the transcriptional level (Kato *et al*., 2005; Tomono *et al*., 2005). In addition to the regulating these proteases, AdpA regulates a *Streptomyces* subtilisin inhibitor (SgiA) that is involved in aerial hyphae formation (Hirano *et al*., 2006), an extracytoplasmic function sigma factor (AdsA) of RNA polymerase (Yamazaki *et al*., 2000), a regulatory protein (AmfR) that is essential for aerial mycelium formation (Yamazaki *et al*., 2003b), and a small acidic protein (SsgA) that is essential for spore septum formation (Yamazaki *et al*., 2003a), constituting an AdpA-regulon (Nishida *et al*., 2007).

In a previous study, we compared extracellular proteins that were partially purified from the culture broths of A-factor-producing *S. griseus* IFO 13350 and from the A-factor-deficient mutant strain *S. griseus* HH1 by using Resource-S cation exchange column chromatography; 4 proteins that were detected only in *S. griseus* IFO 13350 were marked as protein A (33-34 kDa), B (28 kDa), D (9.7 kDa), and E (7-8 kDa), and their N-terminal amino acid sequences were determined. Of them, protein B was identified as SprT encoding *S. griseus* trypsin (SGT); moreover, it was confirmed that AdpA could bind to the promoter region and activate transcription of *sprT* (Kato *et al*., 2005), but the other proteins have not been further studied.

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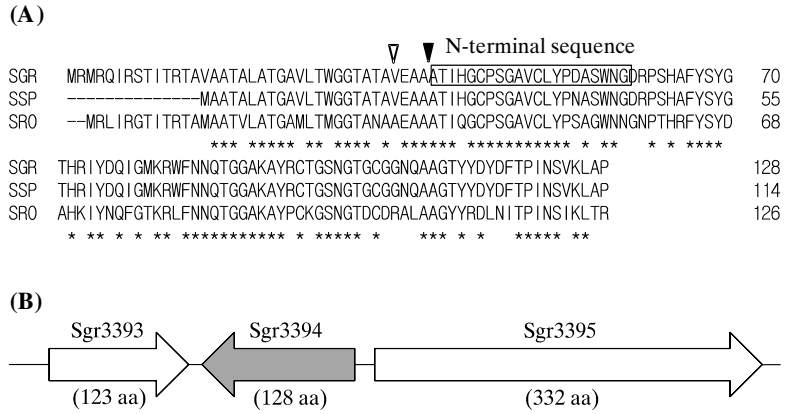

Fig. 1. Characteristic features of Sgr3394. (A) Amino acid alignment between Sgr3394 and its homologues in *Streptomyces*. The primary and secondary cleavage sites and N-terminal sequence are indicated by arrow heads (▽ and ▼) and box, respectively. The perfectly identical amino acids between the 3 proteins are indicated by asterisks. SGR, Sgr3394 of *S. griseus* IFO13350; SSP, SACT1DRAFT-0503 of *Streptomyces* sp. ACT-1; SRO, SrosN15-18634 of *S. roseosporus* NRRL15998. (B) Gene organization of SGR3394. The predicted open reading frames (ORFs) are as follows: SGR3393, hypothetical protein; SGR3394, putative secreted protein; SGR3395, putative ArsR-family transcriptional regulator.

Comparison of the N-terminal amino acid sequence of protein D with the genomic sequence data of *S. griseus* IFO 13350 showed that protein D perfectly matched with Sgr3394, which was annotated only as encoding a putative secretory protein with unknown function. In this paper, we describe the characteristic features of Sgr3394, including regulation of its gene expression in relation to AdpA and its effect on antibiotic production in *Streptomyces*. The materials and methods used in this study, including bacterial strains and plasmids, media and culture conditions, DNA manipulations, purification of protein, and bacterial transformation, were previously described (Kieser *et al*., 2000; Kato *et al*., 2005).

To identify protein D, detected only in *S. griseus* IFO 13350, we compared its N-terminal amino acid sequence (ATIHGXP SGAVXLYPDASWNG (X, not detected)) with the amino acid sequences annotated by Genomic Sequencing Project for *S. griseus* IFO 13350 (Ohnishi *et al*., 2008). Because unmodified cysteine residues cannot be detected by N-terminal protein sequencing via Edman degradation, the unidentified amino acids at the 6th and 12th amino acid positions were deduced to be cysteine residues. When 2 cysteine residues were included, the N-terminal amino acid sequence perfectly matched with the residues between 39 and 49 of Sgr3394 (NCBI Reference Sequence: YP_001824906.1), which encodes a putative secretory protein (Fig. 1A). The premature Sgr3394 consisted of 128 amino acids and showed 87.5% and 68.8% identity with the amino acid sequences of SACT1DRAFT-0503, from *Streptomyces* sp. ACT-1, and SrosN15-18634, from *S. roseosporus* NRRL15998, respectively. An NCBI BlastP search result listed 8 proteins as homologous to Sgr3394 with e-values about $2e^{-5}$, and all of these proteins were annotated as putative secretory proteins. The SignalP 3.0 program (http://www.cbs.dtu.dk/services/SignalP/) predicted 2 cleavage sites of signal peptidase, between Ala^{34} and Val^{35} and between Ala^{37} and Ala^{38} (Nielsen *et al*., 1997). However, because the N-terminal amino acid was determined to be the 39th residue, Sgr3394 should be matured by a second cleavage between the Ala^{38} and Ala^{39} (Fig.

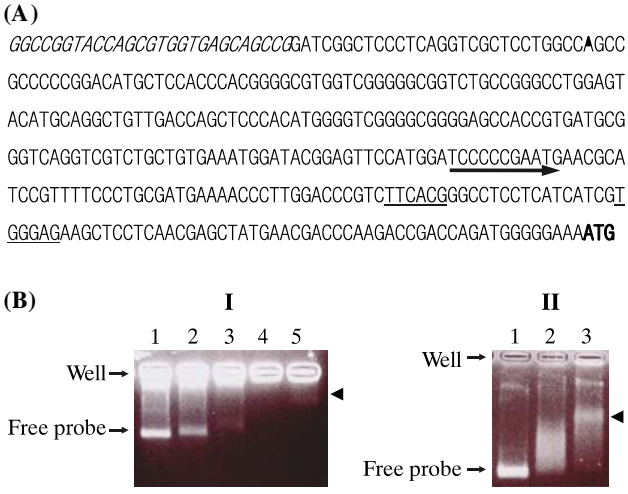

Fig. 2. Gel-mobility shift assay of the *sgr3394* promoter region with AdpA. (A) Nucleotide sequence of the *sgr3394* promoter. The probable -35 and -10 sequences are underlined. The start codon of Sgr3394 and the *sgr3394*-F primer site are indicated by bold and italicized letters, respectively. The arrow indicates the predicted A-factor-dependent transcriptional regulator (AdpA)-binding sequence. (B) Gel-mobility shift assay. (Panel I) Gel-mobility shift assay of the 420-bp DNA probe, including the *sgr3394* promoter region, with AdpA. Lanes: 1, free probe DNA only; 2-5, the probe DNA mixed with 0.4 μg (lane 2), 1.2 μg (lane 3), 2.0 μg (lane 4), and 2.8 μg (lane 5) of AdpA-H (His-tagged AdpA). (Panel II) Gel-mobility shift assay of the *sprT* promoter region, including the AdpA-binding site (probe-T2 covering the region from -112 to +7 in Kato *et al*., 2005), with AdpA as the positive control. Lanes: 1, free probe DNA only; 2 and 3, the probe DNA was mixed with 0.2 μg (lane 2) and 0.4 μg (lane 3) of AdpA-H. The well and free probe and the shifted band are indicated by an arrow and arrow head, respectively. The AdpA-H can bind to the DNA from the *sprT* promoter, forming a clear shifted band, at a low concentration of AdpA-H (0.4 μg); however, more than 6 times that amount AdpA-H (2.8 μg) was necessary to form the shifted band of Sgr3394.

1A). Genomic analysis also showed that *sgr3394* was located between *sgr3393* that encodes a putative protein and *sgr3395* that encodes a putative ArsR-family transcriptional regulator on the opposite strand (Fig. 1B).

Because Sgr3394 was identified only in A-factor-producing *S. griseus* IFO 13350, its expression was assumed to be A-factor dependent, as proven for *sprT*. Therefore, the binding ability of AdpA to the promoter region of *sgr3394* was analyzed by a gel-mobility shift assay. To prepare a DNA probe with a putative AdpA-binding site, an approximately 420-bp DNA fragment was amplified by PCR using *S. griseus* IFO13350 genomic DNA as a template and the following primers (Fig. 2A): Sgr3394-F, 5′-GC*GGAATTC*GGCCGGTACCAGCGTG GTGAG-3′ (nucleotide positions -350 to -323, upstream of the start codon of *sgr3394*, italicized letters indicate an *Eco*RI site); and Sgr3394-GR2, 5′-CCATGTGAGTACGGCGCCGG TGGC-3′ (nucleotide positions +59 to +82, downstream of the start codon of *sgr3394*). The amplified DNA fragment was cloned into a T&A cloning vector, and then the *Hin*dIII-digested DNA fragment was purified using a Gel extraction kit (Dyne Bio, Korea). The AdpA protein, with a His-tag at its C-terminus, was purified using an Ni^{2+}-NTA (nitrilotriacetic acid) column, according to a previously described method (Yamazaki *et al*., 2000), and concentrated by ultrafiltration (10-kDa cutoff). Different concentrations of AdpA were mixed with the DNA probe in a total volume of 20 μl, containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10% glycerol, 0.01% BSA, and 0.0025% poly(dI-dC)-(dI-dC). The mixtures were incubated at 30°C for 30 min, loaded onto a 2% agarose gel, and stained with the SYBR Green EMSA Stain kit (Invitrogen, USA).

The shift of the 420-bp probe DNA, observed in the presence of a high concentration of AdpA-H (Fig. 2A), was different from the distinct band shift of the sprT promoter region observed even at a low concentration of AdpA-H (Fig. 2B). AdpA activates its target genes by binding to the conserved DNA-binding site(s), 5′-TGGCSNGWWY-3′ (S, G, or C; W, A, or T; Y, T, or C; N, any nucleotide), in the region upstream of the transcriptional start point (Yamazaki *et al*., 2004). A predicted AdpA-binding sequence, 5′-TCCCCCGAAT-3′ (nucleotide positions -140 to -131, upstream of the start codon of *sgr3394*), showing homology with the conserved AdpA-binding site, is present upstream of the putative -35 (TTCACG) and -10 (TGGGAG) regions predicted from the consensus -35 (TTGACR) and -10 sequences (TAGRRT; R, A, or G) for housekeeping genes in *Streptomyces* species; however, the probe showed a very weak affinity for AdpA-H. To determine whether the predicted AdpA-binding site was recognized by AdpA, we generated a mutated probe by replacing the original TCCCCC sequence in the putative AdpA-binding site with a *Kpn*I recognition sequence, CTCGAG. Unexpectedly, a gel-mobility shift assay with the mutated probe and AdpA showed a similar shifted band as indicated by the result with the original sequence (data not shown); this finding indicates that AdpA bound nonspecifically to the 420-bp DNA probe. These results clearly show that AdpA cannot bind to the promoter and thus cannot regulate gene expression of *sgr3394* firsthand. Instead, a different protein that is absolutely dependent on A-factor seems to be necessary for expression or translocation of *sgr3394*.

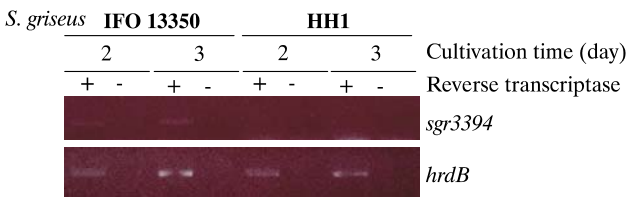

Fig. 3. Transcriptional analysis of *sgr3394* by reverse Transcriptase (RT)-PCR. Total RNAs were extracted, from both *S. griseus* IFO13350 and *S. griseus* HH1 that had been grown for 3 days in YMPD liquid medium, and then used for RT-PCR. As a control for the purity and amount of RNA used, the transcription of *hrdB*, which occurs throughout growth, was analyzed in the same RNA samples.

To prove our assumption, we analyzed the transcript of *sgr3394* by RT-PCR. Total RNAs were extracted from both *S. griseus* IFO13350 and HH1, using the RNAiso kit (TaKaRa shuzo, Japan), from cells grown for 2 or 3 days in YMPD liquid medium. RNA was treated with DNase for 1 h at 37°C, followed by phenol-chloroform extraction. cDNA synthesis was performed using a SuperScript III RT-PCR system (Invitrogen), according to the manufacturer's protocol. RT-PCR without reverse transcriptase was performed as a standard method to test contamination of DNA, and the following primers were used for *sgr3394*: 3394-RTF, 5′-GCGTCAGATTCGGAGCA C-3′ (nucleotide positions +9 to +26, downstream of the start codon); and 3394-RTR, 5′-CGTAGTCGTAGTACGTGC-3′ (nucleotide positions +349 to +332, downstream of the start codon). As the control, RT-PCR was performed using *hrdB*, a housekeeping gene that is constitutively transcribed, with the following primers: hrdB-RTF, 5′-GGCTGATGGGCAGA TCGC-3′ (nucleotide positions +131 to +148, downstream of the start codon); and hrdB-RTR, 5′-CTCGTCGTCGTCG GAGAG-3′ (nucleotide positions +650 to +633, downstream of the start codon). A strong signal was detected for transcription of *hrdB* (control) at a similar level in both the strains; however, transcription of *sgr3394* was detected only in the wild-type strain, at a low level (Fig. 3). Transcription of *sgr3394* was not observed in *S. griseus* HH1. These results strongly suggested that a protein that is expressed only in the wild-type strain, probably in an A-factor-dependent manner, is necessary for transcription of *sgr3394*.

Recently, the effects of A-factor on global gene expression were determined using DNA microarray analysis of transcriptomes in *S. griseus*; 477 genes were found to be differentially expressed during the 12 h after addition of A-factor (Hara *et al*., 2009), but *sgr3394* was not included in the list, and this finding is in agreement with our results. Instead, the expression of *sgr3395*, located upstream of *sgr3394*, was found to be 2.24 times higher within 1 h after A-factor addition than it was in samples with no addition of A-factor. A-factor was also found to induce streptomycin production and resistance (Vujaklija *et al*., 1991). Of the 3 promoters in the streptomycin production region, *strR*, *aphD*, and *strB*, only *strR*, which encodes a regulatory protein, was found to be directly controlled by A-factor. The StrR activator can induce active transcription of other streptomycin biosynthetic genes, including *aphD*, which encodes the major streptomycin resistance

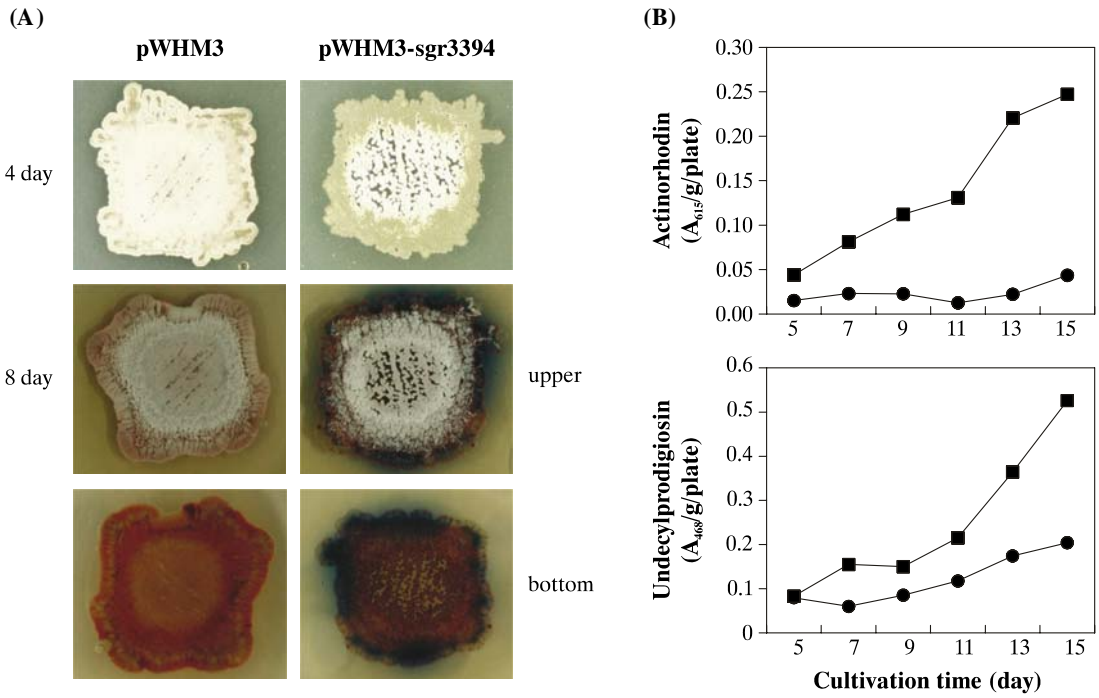

Fig. 4. Gene dosage effect of *sgr3394* on pigment antibiotic production and morphological differentiation by *S. lividans* TK21 on R2YE agar plate. (A) Photographs of *S. lividans* TK21 transformants grown on R2YE agar plate for 4 and 8 days. pWHM3, *S. lividans* with pWHM3 vector only; pWHM3-*sgr3394*, *S. lividans* with pWHM3-*sgr3394*. On the plate of 8-day culture, formation of blue pigment (actinorhodin) around colonies was clearly observed. (B) Production of actinorhodin (upper) and undecylprodigiosin (lower) by *S. lividans* TK21 transformants on R2YE agar plate. A 300-μl aliquot of the cell suspension, prepared from *S. lividans* TK21 transformants, was spread on the surface of cellophane, placed on the R2YE agar medium. After incubation at 28°C for several days, as indicated, the cellophane was removed. The agar was homogenized, heat-melted, and then used for measuring actinorhodin and undecylprodigiosin concentrations at 615 nm (pH 12) and 468 nm (pH 2), respectively. (●) antibiotic from *S. lividans*/pWHM3; (■) antibiotic from *S. lividans*/pWHM3-*sgr3394*.

determinant, streptomycin-6-phosphotransferase. Similarly, it is highly probable that the production of Sgr3395, a putative ArsR-family transcriptional regulator, is induced by A-factor, and Sgr3395 can subsequently act to induce target genes such as *sgr3394*; however, this theory needs further investigation.

We could not find any clues about the function of Sgr3394 from a protein BLAST search because its amino acid sequence shows no identity with functionally well-known proteins. Nevertheless, we expected Sgr3394 to be involved in morphological development or the formation of secondary metabolites in *Streptomyces*, because it was purified from the culture broth of the A-factor-producing wild-type strain, which undergoes complex morphological changes and produces various secondary metabolites, such as streptomycin and grixazone, in contrast to the A-factor-deficient mutant strain HH1 (Nishida *et al*., 2007).

Generally, secondary metabolite formation and morphological differentiation of *Streptomyces* are affected by many factors such as nutrient limitation, environmental change, global regulatory and pathway-specific regulatory factors, and enzymes (Chater, 1993; Chakraburty and Bibb, 1997). Among *Streptomyces* spp., *S. lividans* is a well-known host for heterologous expression of industrially important proteins and compounds (Kim *et al*., 2007). Interestingly, it has biosynthetic gene clusters for production of the pigmented antibiotics, actinorhodin (blue pigment) and undecylprodigiosin (red pigment). However, these gene clusters are present as sleeping genes, and thus, pigmented antibiotics are rarely produced under normal conditions. Many factors mentioned above, particularly the presence of a high concentration of sucrose, can induce active expression of these biosynthetic gene clusters, resulting in massive production of the pigmented antibiotics (Doull and Vining, 1996). In addition, the introduction of many global regulatory genes such as *afsR* (Hong *et al*., 1991), *afsQ* (Ishizuka *et al*., 1992), and *absA* (Aceti and Champness, 1998; Anderson *et al*., 2001), can induce the expression of the sleeping biosynthetic gene clusters and stimulate biosynthesis of actinorhodin and undecylprodigiosin, as well as induce morphological changes in this strain. Therefore, the ability of a putative regulatory gene to induce the production of pigmented antibiotics or morphological changes in *S. lividans* has been routinely used for showing its regulatory function. To confirm the role played by Sgr3394 associated with secondary metabolism or morphological differentiation in the cell, we heterologously overexpressed *sgr3394* in *S. lividans* and analyzed the phenotypic change of the transformant.

The *sgr3394* gene was amplified by PCR using the following primers: *sgr3394*-F, 5′-GC*GGAATTC*GGCCGGTACCAGCG TGGTGAG-3′ (nucleotide positions -350 to -323, upstream of the start codon of *sgr3394*, italicized letters indicate an *Eco*RI site); and *sgr3394*-R, 5′-CGC*AAGCTT*CCGTGCCGG GAGACAGAGCGC-3′ (nucleotide positions +41 to +15,

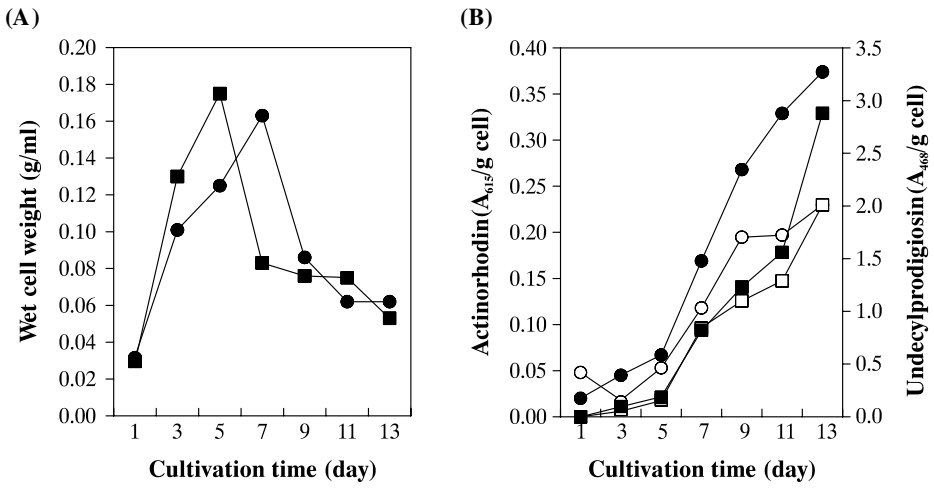

Fig. 5. Effect of *sgr3394* on pigment antibiotic production by *S. lividans* TK21 in R2YE liquid culture. (A) Growth curve. The wet cell weight was estimated at intervals of 2 days and depicted in g/ml of culture broth. (●) wet cell weight of *S. lividans* TK21/pWHM3-*sgr3394*; (■) wet cell weight of *S. lividans* TK21/pWHM3. (B) Production of actinorhodin and undecylprodigiosin by *S. lividans* TK21 transformants. The strains were cultivated at 28°C for 13 days with vigorous shaking. The amounts of actinorhodin and undecylprodigiosin in culture broth were measured at 615 nm (pH 12) and 468 nm (pH 2), respectively, and are represented in absorbance/grams of cell (wet weight). (○) actinorhodin from *S. lividans*/pWHM3; (●) actinorhodin from *S. lividans*/pWHM3-*sgr3394*; (□) undecylprodigiosin from *S. lividans*/pWHM3; (■) undecylprodigiosin from *S. lividans*/pWHM3-*sgr3394*.

downstream of the stop codon, italicized letters indicate a *Hin*dIII site). The amplified DNA fragment was subcloned into a T&A cloning vector (Real Biotech Corp., USA), creating T&A-sgr3394, and was subsequently tested for PCR errors by nucleotide sequencing. The *Eco*RI-*Hin*dIII fragment from T&A-sgr3394 was inserted into a *Streptomyces-Escherichia coli* shuttle vector, pWHM3, that had been digested with the same restriction enzymes, resulting in pWHM3-sgr3394.

When pWHM3-sgr3394 was introduced into *S. lividans* TK21, the transformant produced a massive amount of the blue-pigmented antibiotic (actinorhodin) on a R2YE agar plate (Fig. 4A). Formation of aerial mycelium in *S. lividans* TK21/pWHM3-sgr3394 in the marginal area of the colonies seemed to be slightly poor; however, the overall effect of Sgr3394 on morphological differentiation was negligible. Production of red pigment (undecylprodigiosin) was not visually observed on the plate. To measure the amount of actinorhodin and undecylprodigiosin secreted onto the agar plate, the transformants were precultured at 28°C for 3 days in R2YE liquid medium. The cells were collected by centrifugation and resuspended in the same volume of fresh R2YE liquid medium; subsequently, 300 μl of the cell suspension was spread on the surface of cellophane, placed on the R2YE agar medium. During 15 days of cultivation under the same conditions, the cellophane was removed, at 5, 7, 9, 11, 13, and 15 days, from the agar plate and the cells on the cellophane were weighed. The agar was homogenized, heat-melted, and then used for measuring actinorhodin and undecylprodigiosin concentrations at 615 nm (pH 12) and 468 nm (pH 2), respectively (Kim *et al*., 2007). *S. lividans* TK21/pWHM3-sgr3394 started to synthesize actinorhodin on the seventh day and the amount continuously increased until the 15th day of cultivation, on which the amount synthesized was 6 times higher than the corresponding amount produced by *S. lividans* TK21/pWHM3 (Fig. 4B, Upper). Undecylprodigiosin production showed a similar pattern of increase with prolonged cultivation time. On the last day of measurements *S. lividans* TK21/pWHM3-sgr3394 produced an amount 2.6 times higher than that produced by *S. lividans* TK21/pWHM3 (Fig. 4B, Below).

The gene dosage effect of *sgr3394* on actinorhodin and undecylprodigiosin production was also studied in liquid medium, according to the method previously described by Kim *et al*. (2007). Solid blocks (1 cm × 1 cm) of R2YE agar, grown with the transformant, were cut and used to inoculate 100 ml R2YE liquid medium in 500-ml baffled flasks. The strains were cultivated at 28°C for 13 days with vigorous shaking. Sampling was performed at intervals of 24 h. After centrifugation, the supernatants were used to measure pigmented antibiotics, and the pellets were used to determine cell weight. The amounts of actinorhodin and undecylprodigiosin in the aqueous phase were measured at 615 nm (pH 12) and 468 nm (pH 2), respectively.

The *S. lividans* TK21/pWHM3-sgr3394 showed slower growth than the control and reached maximum cell mass (0.16 g/ml) on the 7th day, whereas *S. lividans* TK21/pWHM3 reached maximum cell mass (0.17 g/ml) on the 5th day of cultivation in R2YE liquid medium (Fig. 5A). However, pigment production started to increase sharply on the seventh day and continued to increase until the 13th day of cultivation. The amounts of actinorhodin and undecylprodigiosin produced by *S. lividans* TK21/pWHM3-sgr3394 on 13th day were 3.1 and 1.4 times higher, respectively, than those produced by the control (Fig. 5B). When the same plasmid was introduced into *S. coelicolor* A3(2), an actinorhodin and undecylprodigiosin producer, it stimulated the production of actinorhodin and undecylprodigiosin to levels 1.8 and 1.1 times higher than the control under the same conditions, respectively (data not shown). However, when the pWHM3-sgr3394 was introduced

into *S. griseus* IFO13350, the transformant did not show any significant changes in morphological differentiation or streptomycin production. Additionally, *sgr3394*-deleted *S. griseus* IFO13350 produced streptomycin and sporulated normally (data not shown).

In conclusion, we identified protein D, which was previously purified from the A-factor-producing *S. griseus* IFO 13350, as Sgr3394, which had been annotated as a putative secretory protein. Although Sgr3394 expression was expected to be directly controlled by A-factor in *via* of AdpA, our results show that it was indirectly controlled at the transcriptional level by a protein positioned downstream of the AdpA-regulatory cascade. Introduction of *sgr3394* on a high-copy-numbered plasmid into *S. lividans* and *S. coelicolor* stimulated antibiotic production and slightly repressed morphological differentiation, suggesting its effect on either typical phenotypic or physiological differentiation in *Streptomyces.* Interestingly, a Blast search showed that Sgr3394 showed high homology with 8 putative secretory proteins, all of which have been reported to occur only in the genus *Streptomyces*. Thus, it is highly possible that Sgr3394 is a *Streptomyces*-specific protein, the function of which is closely related to the characteristic features of the genus *Streptomyces*, such as secondary metabolism and morphological differentiation; however, this theory requires further investigation.

This work was supported by Grant No. 2009-0073015 from the Basic Research Program of the National Research Foundation (KRF) of Korea.

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