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SHORT COMMUNICATION

Two Base Mutations of a Putative LacI-family Transcriptional Regulator, *SCO7554*, Reverse the Overexpression Effect on the Antibiotic Pigment Production in *Streptomyces coelicolor*

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Abstract The *SCO7554* protein, a putative LacI-family transcriptional regulator, is important in the antibiotic pigment production in *Streptomyces coelicolor* M130. A single mutation at the 4th amino acid from asparagine to serine reversed the *SCO7554* effect on the antibiotic pigment production in an overexpression experiment. Additional mutation at the 276th amino acid from valine to alanine intensified this effect, and the overexpression of the double mutant *SCO7554* increased the production of antibiotics, whereas the overexpression of *SCO7554* itself decreased the antibiotic productions. This observation suggested the functional importance of the N-terminal region of a putative LacI-family transcriptional regulator, *SCO7554* protein, even though it was not located on any known functional domain.

Keywords actinorhodin · putative LacI-family transcriptional regulators · *SCO7554* · site-directed mutation · *Streptomyces coelicolor* · undecylprodigiosin

Streptomyces is a well-known genus for producing a broad spectrum of secondary metabolites including antibiotics. Amongst them, *Streptomyces coelicolor* has been used as a model system for the study of *Streptomyces* sp. Understanding of the regulatory systems for gene expression in *S. coelicolor* provides biological approaches to increase the production of valuable bioactive molecules. Many LacI-family transcriptional regulators are involved in primary carbohydrate metabolism (Weickert and Adhya, 1992). Previously, we have studied the overexpression effect of two putative LacI-family transcriptional regulators, *SCO4158* and *SCO7554*, on the production of antibiotics by *S. coelicolor* and *Streptomyces lividans* (Meng et al., 2012). The heterogeneous *SCO7554* overexpression involving the overexpression of *SCO7554* from *S. lividans* in *S. coelicolor* and vice versa, had an opposite effect as compared to the homogeneous overexpression, on the production of antibiotic pigments regardless of strains. This switched effect was an example of the physiological function of codon usage since the difference between *SCO7554* from *S. coelicolor* and *SCO7554* from *S. lividans* was only of two codons without causing any change in the amino acid sequence. During the cloning of *SCO7554* for the overexpression experiment, we accidently introduced mutations in *SCO7554*, thereby leading to a remarkable change in the antibiotic pigment production; we report this change in the present study.

S. coelicolor M130 was used along with *Escherichia coli* DH5αF' and *E. coli* ET12567 as hosts for routine sub-cloning and transformation, respectively. Whereas the R5^{-} medium, which was modified from the R5 medium (without KH_2PO_4, $CaCl_2$, and L-proline), was employed for culturing and measuring the antibiotic production in *S. coelicolor* M130 (Li et al., 2008), the YEME medium was used for protoplast regeneration of *S. coelicolor* (Hopwood et al., 1985). Mycelial growth, spore and protoplast preparations, and transformation of *S. coelicolor* were performed according to to the method reported by Kieser et al. (2000). All transformations for each strain were performed with freshly prepared protoplasts from the same batch of mycelium to

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ensure reproducibility and were repeated at least three times. If necessary, ampicillin was added to the culture media at a concentration of 50 mg/mL and thiostrepton was used at 5 mg/mL.

For the overexpression of *SCO7554*, the entire *SCO7554* gene was amplified by the polymerase chain reaction (PCR) using Ex Taq DNA polymerase (Takara Korea Biomedical Inc., Korea), genomic DNA of *S. coelicolor* as a template, and a pair of primers, namely, 7554-OV-F (5'-CAATCTAGACAGGTCAACT CCGCGGCAGACAGC-3') and 7554-OV-R (5'-CAAAAGCTTC TACGACGACTCGCGCACCACCAG-3'). The amplified PCR products were analyzed by an agarose gel electrophoresis and purified via a DNA extraction kit (Cosmo Genetech Co, Ltd., Korea). The purified PCR product was ligated into pGEM-T (Promega Korea, Ltd., Korea) followed by its complete nucleotide sequencing (Bionics Co., Korea). The PCR-amplified gene was cloned into the *HindIII* and *XbaI* double-digested pWHM3 containing a strong constitutive *ermE* promoter. The constructed plasmids as well as the empty vector pWHM3 were introduced into *S. coelicolor* via PEG-mediated protoplast transformation (Hopwood et al., 1985). The overexpression strains were screened based on antibiotic resistances and confirmed by isolation of plasmids and sequencing.

Interestingly, during screening the plates for the change of antibiotic pigment production, we found two colored phenotypes (Fig. 1). One of the strains showed a bright red color, which indicated a reduced actinorhodin production (OE-1 in Fig. 1), whereas the other strain displayed a stronger darker color pointing to an enhanced actinorhodin production (OE-2 in Fig. 1). We sequenced the cloned genes in pWHM3 from both strains and compared their amino acid sequences (Fig. 2). The OE-1 strain, which harbored *SCO7554*, had the same sequence in the *S. coelicolor* genome. However, the OE-2 strain displayed an altered *SCO7554* at two amino acid residues, namely, asparagine to serine at the 4th position and valine to alanine at the 276th position. Both codon changes occurred by a single base change at the second position, that is, AAC to AGC at the 4th position and GTC to GCC at 276th position.

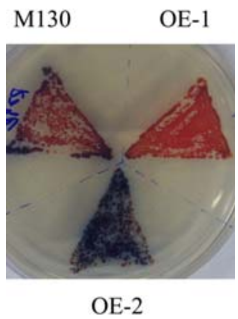

Fig. 1 Transformation in pigment production caused by overexpression of *SCO7554* and mutated *SCO7554* on the R5⁻ agar plate. M130: *Streptomyces coelicolor* M130 as a control; OE-1: *S. coelicolor* M130 with *SCO7554* overexpression; OE-2: *S. coelicolor* M130 with overexpression of mutated *SCO7554*.

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SCO7554   0 MTGNRRPTIK TVAARAGVGR TTVSRVVNGS ELVSADARER VLAAIKELNY VPNSVARGLV TNRTNAVALV  70
OE-1      0 MTGNRRPTIK TVAARAGVGR TTVSRVVNGS ELVSADARER VLAAIKELNY VPNSVARGLV TNRTNAVALV  70
OE-2      0 MTGSRRPTIK TVAARAGVGR TTVSRVVNGS ELVSADARER VLAAIKELNY VPNSVARGLV TNRTNAVALV  70
                *                     Helix-turn-Helix domain

SCO7554  71 IPESESRLGS EPFFAALIRG VSGALAESRT QLQLMLVRDQ AERDQLTASV ATRRVDGVLL VSVHSEDRLP 140
OE-1     71 IPESESRLGS EPFFAALIRG VSGALAESRT QLQLMLVRDQ AERDQLTASV ATRRVDGVLL VSVHSEDRLP 140
OE-2     71 IPESESRLGS EPFFAALIRG VSGALAESRT QLQLMLVRDQ AERDQLTASV ATRRVDGVLL VSVHSEDRLP 140
                                  Putative ligand binding site

SCO7554 141 GMLEEMGLPT VLAGRRDAGE RLSYVNSDNA GGAAAAVRHL LGGGRRRVAT ITGPLDMDVG RSRLAGWRAA 210
OE-1    141 GMLEEMGLPT VLAGRRDAGE RLSYVNSDNA GGAAAAVRHL LGGGRRRVAT ITGPLDMDVG RSRLAGWRAA 210
OE-2    141 GMLEEMGLPT VLAGRRDAGE RLSYVNSDNA GGAAAAVRHL LGGGRRRVAT ITGPLDMDVG RSRLAGWRAA 210
                                  Putative ligand binding site

SCO7554 211 HLEAAVPAEE LLVEAGDFTE EGGASAMRLL LERVPDLDAV FAASDLMAVG ALAELRRQKR QVPGDVAVVG 280
OE-1    211 HLEAAVPAEE LLVEAGDFTE EGGASAMRLL LERVPDLDAV FAASDLMAVG ALAELRRQKR QVPGDVAVVG 280
OE-2    211 HLEAAVPAEE LLVEAGDFTE EGGASAMRLL LERVPDLDAV FAASDLMAVG ALAELRRQKR QVPGDAAVVG 280
                                  Putative ligand binding site                     *

SCO7554 281 FEDSVLARHT NPPLTTVRQP VEELGRTMAR ILTDITQHGA PRQQMTLPTE LVVRESS 337
OE-1    281 FEDSVLARHT NPPLTTVRQP VEELGRTMAR ILTDITQHGA PRQQMTLPTE LVVRESS 337
OE-2    281 FEDSVLARHT NPPLTTVRQP VEELGRTMAR ILTDITQHGA PRQQMTLPTE LVVRESS 337
                                  Putative ligand binding site
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Fig. 2 A comparison between amino acid sequences of *SCO7554* and mutated *SCO7554*. The two altered bases are indicated in bold and an asterisk below the sequence alignment. Helix-turn-helix and putative ligand-binding domains are indicated by range arrows below the sequence alignment.

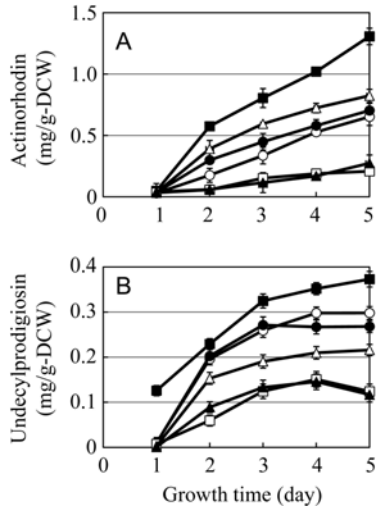

Fig. 3 The effect of mutation on *SCO7554* reflected in the production of antibiotic pigments in an overexpression experiment. Cells were grown in $R5^-$ liquid medium at 28°C with a shaking speed of 250 rpm. ○: *S. coelicolor* M130; ●: *S. coelicolor* M130 with pWHM3; □: *S. coelicolor* M130 with *SCO7554* overexpression; △: *S. coelicolor* M130 with $SCO7554^S$ overexpression; ▲: *S. coelicolor* M130 with $SCO7554^A$ overexpression; ■: *S. coelicolor* M130 with $SCO7554^{SA}$ overexpression.

To characterize the effect of both mutations independently, each mutation was introduced separately into *SCO7554,* and the change in production of the antibiotic pigment by overproduction (Fig. 3) was measured. For the construction of a single-site directed mutation, a two-step PCR was employed using Pfu DNA polymerase (Promega Korea, Ltd, Korea) and four additional internal primers, Asp-Ser-F (5'-GTTGGACGGCGGCTGCCGGTC-3'), Asp-Ser-R (5'-GACCGGCAGCCGCCGTCCAAC-3'), Val-Ala-F (5'-GACGACGGCGGCGTCCCCG-3'), and Val-Ala-R (5'-CGGGGACGCCGCCGTCGTC-3'). The mutation sites are underlined in the sequences for more clarity/better visibility. Further cloning and transformation steps were carried out by constructing the overexpression strain described earlier. Upon overexpressing $SCO7554^A$ (closed triangle in Fig. 3), the actinorhodin and undecylprodigiosin production was similar to that shown by the strain with the *SCO7554* overexpression (opened square in Fig. 3), which in turn was less than half of that of *S. coelicolor* M130 (opened circle in Fig. 3). However, upon overexpressing $SCO7554^S$ (opened triangle in Fig. 3), the extent of antibiotic pigment production changed mildly. This result suggested that any single mutation alone cannot produce the unique phenotype of the double mutation $SCO7554^{SA}$ (closed square in Fig. 3).

In the present study, we have shown that the overexpression of $SCO7554^{SA}$ in *S. coelicolor* resulted in switching of the physiological effect from that of the *SCO7554* overexpression, which could have occurred as a consequence of two amino acid changes in the *SCO7554* protein. Although the exchange of asparagine with serine at the 4th amino acid took placed outside the helix-turn-helix domain, it still interfered with the biological function of the overexpressed *SCO7554* protein, which illustrated the importance of the 4th amino acid, asparagine, in the functioning of the *SCO7554* protein although it was not located at any known domain. Despite the finding that the mutation from valine into alanine took placed in the putative ligand-binding domain at the 276th amino acid, the change in the antibiotic pigment productions by the $SCO7554^A$ overexpression was similar to that by *SCO7554* overexpression, indicating that the mutation into alanine at the 276th amino acid alone did not have a significant effect on the antibiotic pigment production.

Another plausible explanation was offered by the effect of codon usage. In a previous study involving overexpression of a mutated *SCO7554*, two degenerate base changes, which consequently did not reflect a change in the amino acids, enhanced the antibiotic pigment production (Meng et al., 2012). The altered protein synthesis and/or posttranslational modification brought about by the two codon changes in the present study may also have contributed to the switched physiological function of *SCO7554* protein. However, the codon usage analysis of *S. coelicolor* showed that there was no significant change in the usage frequency, according to the genomic analysis (Nakamura et al., 2000), 16.3% of AAC for asparagine to 12.5% of AGC for serine and 46.9% of GTC for valine to 78.4% of GCC for alanine. This codon-usage frequency data may eliminate the possibility of change in the antibiotic pigment production by altered protein translation speed.

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