

Sigma-Factor-Like Global Regulatory Gene, *afsR2* that Stimulates Antibiotic Production in *Streptomyces lividans*

Chang-Young Kim, Hyun-Joo Park, Mi-Yeon Lee, Eung-Soo Kim*
School of Chemical Engineering and Biotechnology, Inha University

Abstract

The 63-amino-acid encoding *afsR2* is a global antibiotics-stimulating regulatory gene originally identified from the chromosome of *Streptomyces lividans*. Although the over-expression of *afsR2* in *S. lividans* significantly induced the production of a deep-blue pigmented antibiotics called actinorhodin, the functional domain and regulatory mechanism of this small-sized *afsR2* gene is still unknown. To dissect a putative functional domain in *afsR2*, various *afsR2*-derivative deletion constructs were generated and screened for the loss of actinorhodin-stimulating capability. The *afsR2*-derivative construct without a 100-bp C-terminal region significantly lost its actinorhodin-stimulating capability in *S. lividans*, implying the presence of critical functional domain located in the C-terminal region of *afsR2*. Three alternating aspartic acids (Leu-Asp-Leu-Asp-Gly-Asp) located in the middle of 100-bp C-terminal region were replaced by three alanines (Leu-Ala-Leu-Ala-Gly-Ala) using site directed mutagenesis. This site-directed-mutant *afsR2* construct failed to stimulate actinorhodin production in *S. lividans*, suggesting that three alternating aspartic acids located in the C-terminal region of *AfsR2* should play a critical functional role as a positive regulatory protein.

The bacterial genus *Streptomyces* is widely known for its ability to produce a variety of secondary metabolites, including medically important products such as antibiotics, antitumor agents, immunosuppressors, and enzyme inhibitors. It has been well documented that antibiotic production generally occurs during the stationary phase of growth of *Streptomyces* spp. cells and correlates temporally with the formation of aerial mycelium in cultures grown on the surface of solid media. Several pleiotropic genes that govern antibiotic production have been identified; some of these affect only antibiotic production whereas others affect both antibiotic production and morphological differentiation, suggesting that the two processes share elements of genetic control.

Among several known regulatory genes affecting antibiotic biosynthetic pathways in *Streptomyces* spp. is *afsR2*, which is also known as *afsS* in *S. coelicolor* and is located immediately 3' to *afsR*, encodes a 63 amino acid protein of unknown function. As the biosynthesis of both actinorhodin and undecylprodigiosin can occur in the absence of *afsR*, this gene is not required for antibiotic production. Multiple copies of *afsR* stimulate overproduction of actinorhodin and undecylprodigiosin

