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

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## **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 (Leu-Ala-Leu-Ala-Gly-Ala) using directed mutagenesis. 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

in both *S. lividans* and *S. coelicolor*, as was observed also for *afsR*. However, whereas a single copy of the *afsR2* gene normally does not lead to significant actinorhodin production in *S. lividans*, one chromosomal copy of the identical gene results in extensive biosynthesis of actinorhodin pigment in *S. coelicolor*, giving *S. coelicolor* colonies the deep blue color responsible for its species name. Recently, it was reported that the expression of *afsR2* in *S. lividans* is physiologically regulated and that *afsR2* mRNA synthesis from a single chromosomal *afsR2* gene can be stimulated by specific growth conditions to yield a *S. coelicolor*-like level of actinorhodin biosynthesis. It also indicated that the requirement for multiple copies of *afsR2* to promote actinorhodin production in *S. lividans* is conditional, rather than absolute, also demonstrate the existence in *S. lividans* of both *afsR2*-dependent and independent mechanisms of actinorhodin synthesis. Unfortunately, however, the functional domain and regulatory mechanism of this small-sized *afsR2* gene is still unknown. Here we report that a 100-bp C-terminal region in *afsR2* is functionally important for actinorhodin-stimulating capability in *S. lividans*. In addition, three alternating aspartic acids (Leu-Asp-Leu-Asp-Gly-Asp) located in the middle of 100-bp C-terminal region of AfsR2 should play a critical functional role as a positive regulatory protein.

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RppD, SigA	(JHF-41-)	FETAEDWO	TPEAVE-118	: AQHPVSÇETP	£u\$H¢L\$H
RpoD	:OPERTA-	HIBELAERYLH	HMPEOKIRI VLK	AKEP SPETE	1030E08H
11:43	:GFFF1-		MEPHAVI - VOK		
H <del>o</del> lA	EGCENTA	EVAAH D	JAPERVG-VLA	LAGENSE-4F	Mê-Boka
AGH)	11-146 (100)	KTHERMITTI ON	HMPSGPAN-ATT	тислнурдарда	L. G.GK
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Fig. 1. Alignment of AfsR2 with region 3 of sigma70 family proteins from *Bacillus* (RpoD, SigA), *E. coli* (RpoD) and *Streptomyces* (HrdA, HrdB, AfsR2)

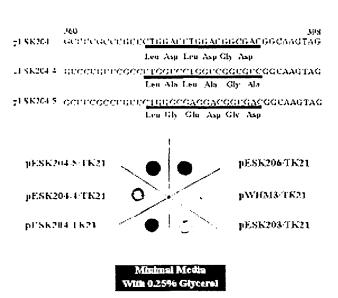


Fig. 2. Site-directed mutagenesis of afsR2 C-terminal region