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Function of S-adenosylmethionine on the Antibiotic Production in Streptomycetes

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S-adenosylmethionine (SAM) is an essential molecule functioning as the major methyl donor in all living organisms. Despite extensive research in many organisms, its role in *Streptomyces* sp. remains unclear. Recently, in our study, the putative SAM-s gene was isolated from a spectinomycin producer, *Streptomyces spectabilis*. The overexpression of the SAM-s gene in *Streptomyces lividans* TK23 inhibited sporulation and aerial mycelium formation, but enhanced the production of actinorhodin. Exogenous treatment of SAM using various streptomycetes resulted in increased antibiotic production. The mechanism by which SAM increased actinorhodin production in *S. coelicolor* may involve the interaction of SAM with serine/theronine protein kinase system. Altogether, our results suggested that SAM is a novel intracellular signal molecule for both cell differentiation and antibiotic production in streptomycetes.

Accumulation of S-adenosyl-L-methionine enhances production of actinorhodin but inhibits sporulation in *Streptomyces lividans* TK23

S-Adenosyl-L-methionine synthetase (SAM-s) catalyzes the biosynthesis of SAM from ATP and L-methionine. In the present study, the putative SAM-s gene was isolated from a spectinomycin producer, *Streptomyces spectabilis*. The purified protein from the transformed *Escherichia coli* with the isolated gene synthesized SAM from L-methionine and ATP in vitro, strongly indicating that the isolated gene indeed encoded the SAM-s protein. The overexpression of the SAM-s gene in *Streptomyces lividans* TK23 inhibited sporulation and aerial mycelium formation but enhanced the production of actinorhodin in both agar plates and liquid media [FIG.1]. Surprisingly, the overexpressed SAM was proven by Northern analysis to increase the production of actinorhodin through the induction of *act*II-ORF4, a transcription activator of actinorhodin biosynthetic gene clusters [FIG. 2]. In addition, we found that a certain level of intracellular SAM is critical for the induction of antibiotic biosynthetic genes, since the control strain harboring only the plasmid DNA did not show any induction of *act*II-ORF4 until it reached a certain level of SAM in the cell. From these results, we concluded that the SAM plays important roles as an intracellular factor in both cellular differentiation and antibiotic production in *Streptomyces* sp.

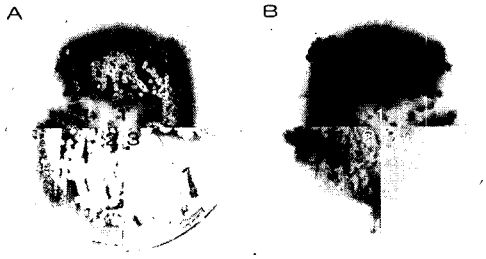

FIG. 1. Expression of the SAM-s gene from *S. spectabilis* enhances actinorhodin production but inhibits sporulation.
(A) The front side of the plate showing sporulation; (B) the reverse side of the plate showing actinorhodin production. Subpanels: 1, cells with SAM-s gene overexpression; 2, cells with empty vector (pWHM3); 3, wild-type S. lividans TK23 cells.

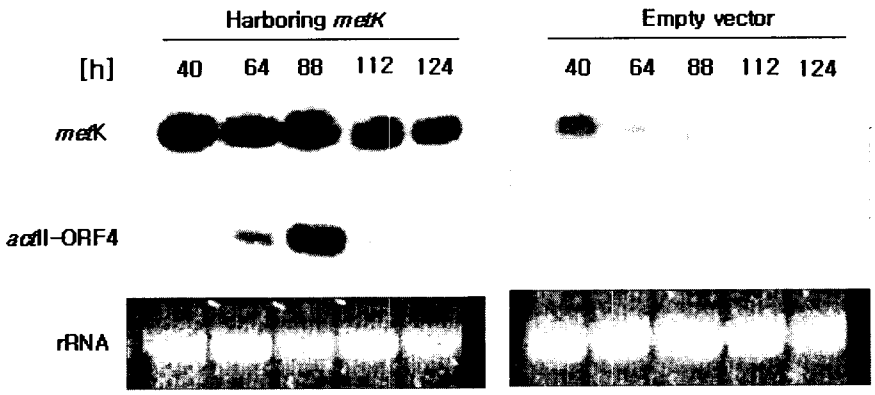

FIG. 2. Introduction of SAM-s elevates the intracellular level of SAM, which in turn activates the transcription of the *act*II-ORF4. Northern analysis of the *act*II-ORF4 gene in cells with or without the SAM-s gene.

Widespread activation of antibiotic biosynthesis by S-adenosylmethionine in streptomycetes

Our studies revealed that accumulation of intracellular SAM enhanced actinorhodin production in *Streptomyces lividans* although SAM-dependent methylation is not involved in its biosynthetic pathway. In this study, the effect of SAM on production of various antibiotics was investigated in different producers in relation to whether SAM-dependent methylation is required in their biosynthetic pathways. Pristinamycin II_B and granaticin contain methyl groups that do not originate from SAM-dependent methylation, and production of these two antibiotics was increased about 2-fold when a low concentration (50 μM and 10 μ M, respectively) of SAM was added; whereas oleandomycin and avermectin B1a contain methyl groups generated from SAM-dependent methylation, and production of these two antibiotics was increased 5-fold and 6-fold depending on SAM concentration in a certain range [FIG. 3]. We also found that transcription of pathway-specific regulator *gra*-ORF9 was activated by exogenous SAM treatment. Oleandomycin and avermectin B1a production was decreased by sinefungin, a methylation inhibitor, and their production was restored to control levels by adding SAM simultaneously with sinefungin. Our results suggested that SAM may regulate antibiotic production via different regulatory mechanisms, either as an intracellular factor or as a cofactor in stimulating antibiotic biosynthesis. It is also possible that these two mechanisms exist

together in one producer, and in this way SAM dramatically increases antibiotic production in some producers. Our studies strongly demonstrated the widespread activation of antibiotic production by SAM in streptomycetes. The enhancement effect of SAM on antibiotic production also implies its significance for yield improvement in industrial applications.

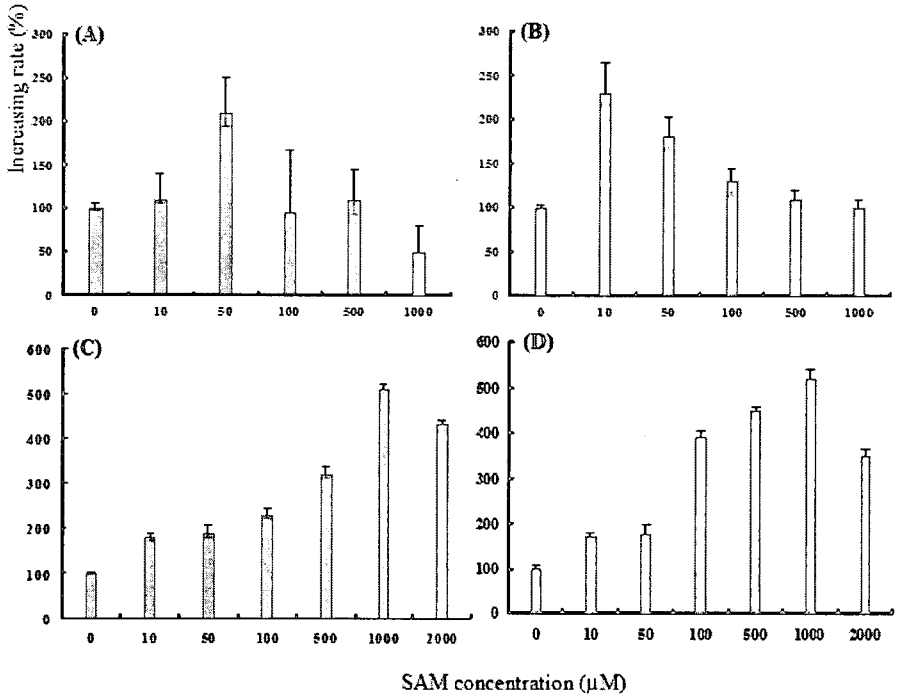

FIG. 3. Concentration effect of SAM on antibiotic production. Concentration effect of SAM on (A) pristinamycin, (B) granaticin, (C) oleandomycin, and (D) avermectin production. Production of pristinamycins and granaticin was increased by exogenous treatment of SAM with the optimal concentration of 50 μM and 10 μM, respectively. Production of oleandomycin and avermectin was increased about 5-fold respectively by SAM in a dosage-dependent manner in the concentration range 100 μM to 1 mM while in low concentration at 10 μM and 50 μM their production was increased about 2-fold.

S-adnosylmethionine activates actinorhodin biosynthesis by interacting with serine/threonine protein kinase AfsK and increasing its phosphorylation in *S. coelicolor* M130

The mechanism of SAM in enhancing actinorhodin production in *S. coelicolor* was studied. Mutants from the different stages of AfsK/AfsR serine/theronine protein kinase signaling cascade was used to test the possible interaction with SAM. The results showed that no effect of SAM on actinorhodin production could be observed in *afs*K and *afs*R mutants, while at the same time SAM slightly increased actinorhodin production in *afs*S mutant. The putative SAM binding motifs in AfsK led us to further investigate the interaction of SAM with this protein kinase. We found that SAM increased the phosphorylation of kinase AfsK [FIG. 4], what's more, decrease of actinorhodin production by a serine/theronine kinase inhibitor K252a can be circumvented by SAM. Our results demonstrate that SAM activates actinorhodin

biosynthesis in *S. coelicolor* M130 by interacting with protein kinase AfsK and increasing its phosphorylation. Based on these results, a new model was developed to illustrate the cross-talk of SAM with AfsK/AfsR protein kinase signaling cascade [FIG. 5]. First, binding of SAM on AfsK protein induces a conformational change of this protein kinase. The consequent allosteric change then leads to the high phosphorylation level of this Ser/Thr kinase. Phosphorylation of AfsR is subsequently activated and the phosphorylated form of AfsR then activates the transcription of AfsS. The high level expression of AfsS then induces a higher transcription of *act*II-ORF4, and finally resulting in the overproduction of actinorhodin.

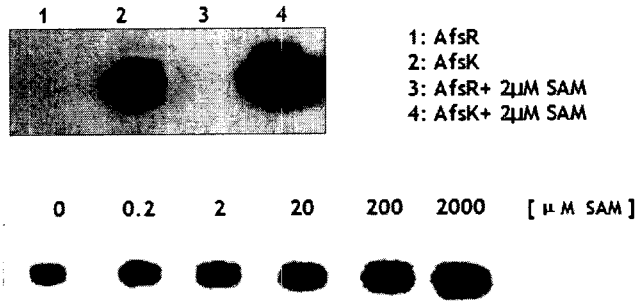

FIG. 4. SAM enhanced phosphorylation of kinase AfsK in vitro. Phosphorylation of AfsK(lane1) was enhanced by 2μM SAM (lane 4). And phosphorylation of AfsR by AfsK(lane 5) was also increased (lane 6). The activation of phosphorylation by SAM presented a dose-dependent manner.

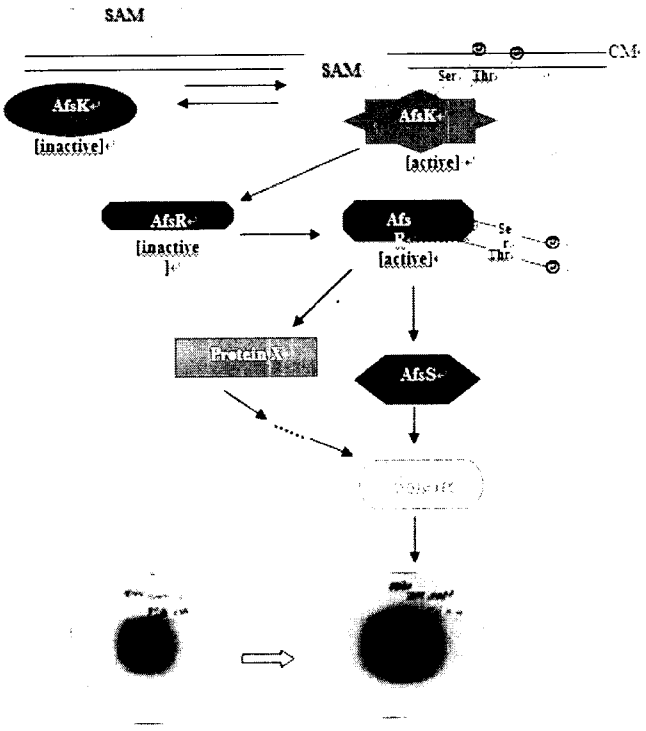

FIG. 5. Proposed model for interaction of SAM with AfsK/AfsR regulatory cascade modified from the model developed by Horinouchi group.

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