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S-Adenosyl-Methionine, a Novel Intracellular Factor for Both Cell Differentiation and Antibiotic Production in Streptomycetes

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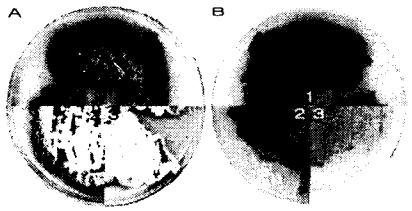
S-adenosylmethionine (SAM) is an essential molecule functioning as the major methyl donor in all living organisms. Recently, our studies revealed that accumulation of intracellular SAM affected both cell differentiation and antibiotic production in *Streptomyces lividans*. Exogenous treatment of SAM 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 AfsK/AfsR protein kinase system. Altogether, our results suggested that SAM is a novel intracellular signal molecule for both cell differentiation and antibiotic production in streptomycetes.

SAM is the major methyl donor in all living organisms. However, recently, we found that overexpression of SAM-synthase in *S. lividans* enhanced antibiotic production but inhibited spore differentiation in *Streptomyces lividans* TK23, although SAM-dependent methylation is not involved in actinorhodin biosynthetic pathway (Fig. 1). At the same time, transcription of pathway-specific gene *actII*-orf4 was elevated (Fig. 2).



A, front side, showing spore formation, B, reverse side, showing antibiotic production. 1, *S. lividans* harboring plasmid with SAM-s gene, 2, *S. lividans* haboring empty vector, 3, wild type *S. lividans*.

Fig. 1 Antibiotic production and cell differentiation by overexpression of SAM-synthase gene.

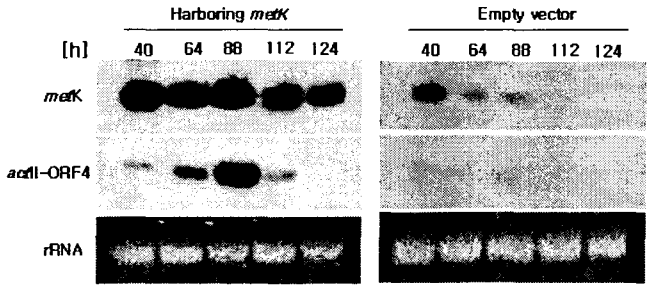


Fig. 2 Transcription of *actII-orf4* by overexpression of SAM-synthase gene (*metK*)

To examine the possibility that SAM exerted its regulatory effect as an intracellular factor besides its role as cofactor of methylation, the effect of SAM on the production of various antibiotics was investigated in different antibiotic producers, in relation to whether SAM-dependent methylation is required in their biosynthetic pathway. Pristinamycin II_B and granaticin contain methyl groups that are not originated from SAM-dependent methylation, and production of these two antibiotics was increased about 2-fold when a low concentration (50 μM and 10 μM respectively) of SAM was added (Fig. 3, a and b); whereas oleandomycin and avermectin B1a contain methyl groups generated from SAM-dependent methylation, and production of these two antibiotics was increased 5-fold and 7-fold depending on SAM concentration in a certain range (Fig. 3, c and d). We also found that transcription of pathway-specific regulator *gra*-ORF9 was activated by exogenous SAM treatment (data not shown). The widespread activation of antibiotic production by SAM clearly demonstrated that besides its role as methyl donor, SAM also functions as an intracellular signaling molecule to induce secondary metabolism in streptomycetes.

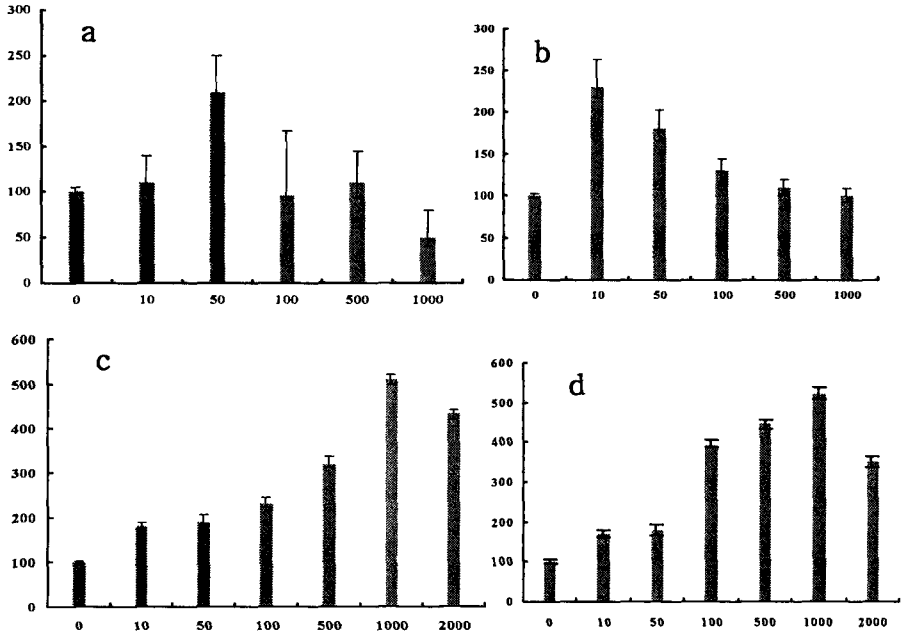


Fig. 3 Widespread activation of antibiotic production by SAM a, Pristinamycin, b, granaticin, c, olendomycin, d, avermectin production.

The mechanism of SAM in enhancing actinorhodin production was studied in *S. coelicolor*. 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 *afsK* and *afsR* mutants, while at the same time SAM slightly increased actinorhodin production in *afsS* 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, what's more, decrease of actinorhodin production by a serine/theronine kinase inhibitor K252a can be circumvented by SAM (data not shown). Based on the above results, a new model was proposed concerning the interaction of SAM with AfsK/AfsR protein kinase regulatory cascade (Fig. 4).

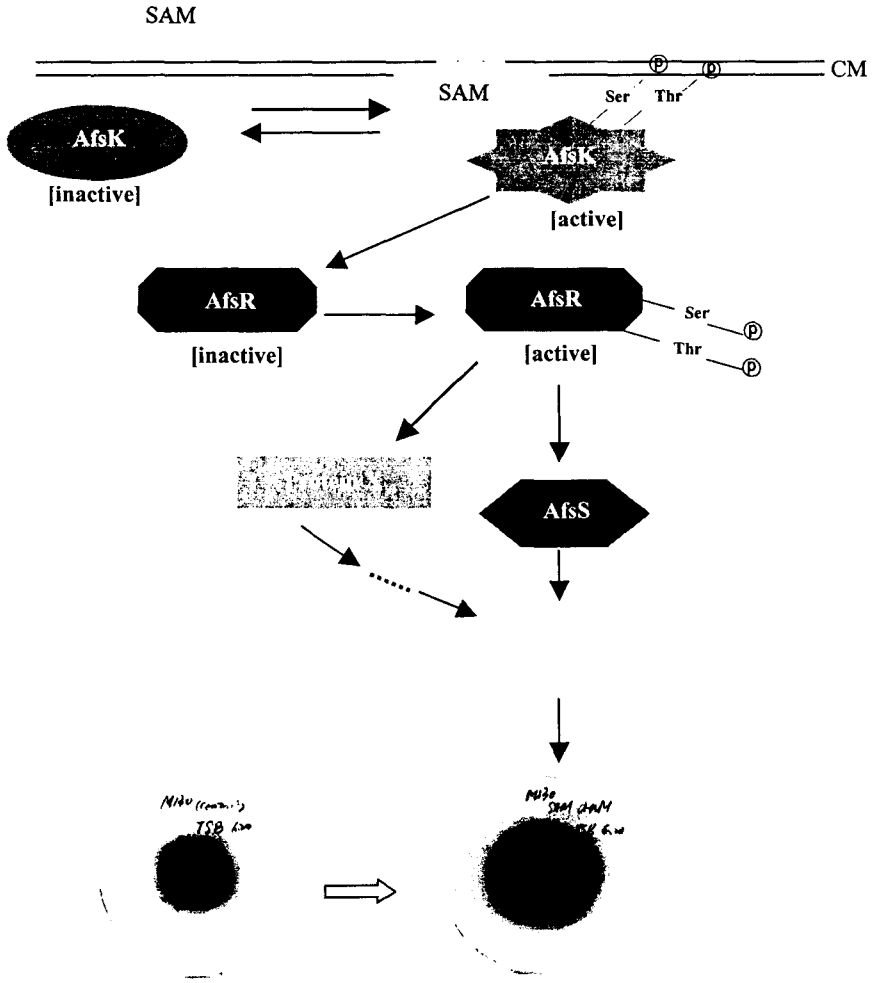


Fig. 4 Proposed model for interaction of SAM with AfsK/AfsR regulatory cascade modified from the model developed by Horinouchi group.

References

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