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# Role of adenosine kinase in the control of *Streptomyces* differentiations: Loss of adenosine kinase suppresses sporulation and actinorhodin biosynthesis while inducing hyperproduction of undecylprodigiosin in *Streptomyces lividans*

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**Abstract**

Adenosine kinase (ADK) catalyses phosphorylation of adenosine (Ado) and generates adenosine monophosphate (AMP). ADK gene ($adk_{Sli}$, an ortholog of *SCO2158*) was disrupted in *Streptomyces lividans* by single crossover-mediated vector integration. The $adk_{Sli}$ disruption mutant ($\Delta adk_{Sli}$) was devoid of sporulation and a plasmid copy of $adk_{Sli}$ restored sporulation ability in $\Delta adk_{Sli}$, thus indicating that loss of $adk_{Sli}$ abolishes sporulation in *S. lividans*. Ado supplementation strongly suppressed sporulation ability in *S. lividans* wild-type (*wt*), supporting that disruption of $adk_{Sli}$ resulted in Ado accumulation, which in turn suppressed sporulation. Cell-free experiments demonstrated that $\Delta adk_{Sli}$ lacked ADK activity and *in vitro* characterization confirms that $adk_{Sli}$ encodes ADK. The intracellular level of Ado was highly elevated while the AMP level was significantly reduced after loss of $adk_{Sli}$ while $\Delta adk_{Sli}$ displayed no significant derivation from *wt* in the levels of *S*-adenosylhomocysteine (SAH) and *S*-adenosylmethionine (SAM). Notably, Ado supplementation to *wt* lowered AMP content, albeit not to the level of $\Delta adk_{Sli}$, implying that the reduction of AMP level is partially forced by Ado accumulation in $\Delta adk_{Sli}$. In $\Delta adk_{Sli}$, actinorhodin (ACT) production was suppressed and undecylprodigiosin (RED) production was dramatically enhanced; however, Ado supplementation failed to exert this differential control. A promoter-probe assay verified repression of *actII-orf4* and induction of *redD* in $\Delta adk_{Sli}$, substantiating that unknown metabolic shift(s) of ADK-deficiency evokes differential genetic control on secondary metabolism in *S. lividans*. The present study is the first report revealing the suppressive role of Ado in *Streptomyces* development and the differential regulatory function of ADK activity in *Streptomyces* secondary metabolism, although the underlying mechanism has yet to be elucidated.


*Keywords:* *Streptomyces lividans*; Adenosine kinase; Adenosine accumulation; Suppression of sporulation; Differential control on secondary metabolism

Adenosine kinase (ADK) (EC 2.1.1.20) catalyses phosphorylation of adenosine (Ado) to adenosine monophosphate (AMP) by using adenosine triphosphate (ATP) as a main phosphoryl donor. An evident intracellular route to Ado is the *S*-adenosylmethionine (SAM) cycle, in which *S*-adenosylhomocysteine (SAH) is hydrolyzed into Ado and L-homocysteine by SAH hydrolase (SAHH) [1]. Ado is then converted into AMP by ADK and L-homocysteine is recycled into L-methionine or enters the transsulfuration pathway. SAM synthetase couples L-methionine with the Ado moiety of ATP to generate SAM. SAM-dependent transmethylation reactions participate in numerous biological processes. While they widely differ in the nature of their substrates they have a common feature in that they all produce SAH and are inhibited by SAH. Therefore, intracellular SAH level is controlled by continuous removal. The SAHH reaction is reversible in physiological conditions,

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whereby Ado accumulation can potentially inhibit the hydrolysis of SAH by SAHH [1]. ADK activity has therefore been asserted to play a role in the prevention of Ado accumulation and support the metabolic cycling of Ado [1–3].

*Streptomyces* is a prolific source of secondary metabolites and many of these compounds have important applications in human medicine. *Streptomyces* is also known for its complex life cycle, which involves a developmental transition from vegetative growth into aerial hyphae formation and then into spores [4]. The present study reveals that an ADK-deficient mutant of *Streptomyces lividans* loses its sporulation ability without being perturbed at the intracellular SAH level. Furthermore, the mutation induces repression in actinorhodin (ACT) biosynthesis and hyperproduction of undecylprodigiosin (RED). The present data substantiates that Ado accumulation, induced by a loss of ADK activity, negatively controls morphological differentiation of *S. lividans*, and unknown metabolic shift(s) of ADK-deficiency differentially regulates ACT and RED biosyntheses.

## Materials and methods

*Bacterial strains, culture conditions and general procedures.* *Streptomyces lividans* TK23 (*spc-1* SLP2$^-$ SLP3$^-$) was used in this study. *Escherichia coli* DH5α and *E. coli* BL21 (DE3) were used as a host for routine subcloning work and a host for protein expression, respectively. R2YE agar was used for maintenance and protoplast regeneration of *S. lividans* TK23 [5]. R2YE agar culture was also used for inspection of sporulation and for determination of intracellular metabolites. The Δ*adk*$_{Sli}$ strain was maintained and cultivated with inclusion of apramycin at 50 μg/ml. Protoplast transformation was conducted according to a previously documented procedure [5]. Determination of catechase (XylE) activities was performed in a crude protein extract as previously described [6]. For determinations of ACT and RED production, one day-old R2YE liquid culture was used to initiate a defined medium culture at 0.05% (v/v) of inoculum size. The composition of the defined medium was previously documented [7]. ACT and RED contents were determined as previously described [5].

*Plasmid construction.* Synthetic oligonucleotide primers are listed in Table 1. For the purpose of gene disruption, a 600-bp fragment internal to *adk*$_{Sli}$ was amplified by PCR from genomic DNA of *S. lividans* by using the oligonucleotide primers AK-xF and AK-xR. The PCR product was digested with EcoRI and HindIII and subcloned into the same sites of pKC1139 to generate pJWS4011. *E. coli-Streptomyces* shuttle vector pKC1139 multiplies by a temperature sensitive replicon in *Streptomyces* [5]. For the gene complementation construct, AKF and AKR primers were used to amplify the full length *adk*$_{Sli}$, which was subcloned into the XbaI and HindIII sites of pWHM3-*ermE*p, generating pJWS4012 (for convenience, termed pADK hereafter). The shuttle plasmid pWHM3 contains *E. coli* pUC19 replicon and *Streptomyces* pIJ101 replicon [5]. *Streptomyces* expression plasmid pWHM3-*ermE*p is a derivative of pWHM3 with a 279-bp KpnI–BamHI *ermE* promoter (*ermE*p) fragment [8] and the plausible ribosomal binding site overlaps with the BamHI site (GenBank Accession No. M11200). For expression of the *adk*$_{Sli}$ product (ADK$_{Sli}$) in *E. coli*, *adk*$_{Sli}$ was amplified with AKFe and AKR primer pairs and ligated into pGEM-T vector (Promega). From the resulting construct, the insert was rescued as EcoRI–NotI fragment and ligated into the same sites of pGEX-5X-1 (Pharmacia) to give pJWS4013. Promoter-probe plasmids were prepared by cloning each promoter segment at the upstream of promoter-less *xylE* in pIJ4083 [9]. Each promoter sequence was amplified by PCR from the chromosomal DNA of *S. lividans* with primers designed on a previously defined promoter sequence in the *S. coelicolor* genome [10,11] (Table 1).

*ADK assay with CFE and recombinant ADK*$_{Sli}$. Cell paste from a R2YE liquid culture at the mid-logarithm phase was used to prepare CFE. Mycelium was collected by centrifugation, and then resuspended in standard buffer (10 mM Tris–Cl, pH 8.0, 1 mM DTT, 10% glycerol) to be sonicated for cell lysis. The supernatant was collected by centrifugation (10,000*g*, 30 min, 4 °C) and subjected to ammonium sulfate precipitation at 60% saturation. A crude protein mixture was collected in pellet form after centrifugation (10,000*g*, 30 min, 4 °C) and resuspended in a minimal volume of the standard buffer to be dialyzed against the same buffer. The resulting CFE was included as 0.5 mg of crude protein per milliliter of ADK assay mixture, which was composed of 50 mM Tris–Cl (pH 7.5), 5 mM $MgCl_2$, 2.5 mM ATP, and 20 μM Ado. The reaction was allowed to proceed for 60 min at 37 °C. For a radiometric assay, each reaction was supplemented with 1.0 μCi [γ-$^{32}$ P] ATP. One microliter of each reaction sample was spotted onto polyethyleneimine-cellulose TLC plate (Merck), which was developed in aqueous 1 M acetic acid and then in aqueous 1.2 M lithium chloride solution [12]. A phosphoimage analyzer (Fuji) was used to monitor the radioactivity of the samples. Formation of AMP in the ADK assay was also monitored by HPLC on the Eclipse XDB-C18 column (4.6 × 250 mm, 5 m, 80 Å, Agilent Technology). The column was first eluted in 20 mM ammonium acetate, pH 5.8, for 5 min, followed by a linear gradient from 0% to 50% acetonitrile in 20 mM ammonium acetate, pH 5.8, for 10 min and maintained at 50% acetonitrile in 20 mM ammonium acetate for an additional 10 min, with a flow rate of 0.8 ml/min and detection at 260 nm. The product of *adk*$_{Sli}$ (ADK$_{Sli}$) was obtained as a recombinant protein tagged with glutathione *S*-transferase (GST) from *E. coli* BL21 (DE3) with pJWS4013 (pGEX-5X-1 harboring *adk*$_{Sli}$). Induction was accomplished with 1 mM IPTG and continued for 4 h at 30 °C. GST-ADK$_{Sli}$ was purified using a GSTrap column following the manufacturer's protocol (Amersham). To confirm that non-tagged ADK$_{Sli}$ retains ADK activity, one milligram of GST-ADK$_{Sli}$ was treated

Table 1
Synthetic oligonucleotides used in this study

| Primer | 5′→3′ Sequence[a] | Description | Relevant feature |
|---|---|---|---|
| AK-xF | ATTAAGCTTAGCTCGGCACCC | +218 to +817 | This work |
| AK-xR | CTTGAATTCAGGCGTCGCCGA | | |
| AKF | CTTTCTAGAGTGAACCGTCAACCC | +1 to +1017 | This work |
| AKFe | CTTGAATTCGTGAACCGTCAACCC | | |
| AKR | ATTAAGCTTTCAGCCCAGGTGCGG | | |
| *act*-pF | CTTAAGCTTATAGGAGATCGC | −96 to +537 | *actII-orf4* promoter [10] |
| *act*-pR | ATTTCTAGATCTGCCCAGCCG | | |
| *redD*-pF | CTTGAATTCATCTTCCTCTTT | −215 to +82 | *redD* promoter [11] |
| *redD*-pR | ATTGGATCCCGGGGTGCCGCC | | |

Description: the positions relative to putative translation start codon.
[a] Underlined nucleotides indicate nonhomologous sequences added to create restriction sites.