SUPPLEMENTARY MATERIAL

Acetoacetyl-CoA synthetase activity is controlled by a protein acetyltransferase with unique domain organization in *Streptomyces lividans*

Alex C. Tucker[1] and Jorge C. Escalante-Semerena[2]*
[1]Department of Bacteriology, University of Wisconsin
1550 Linden Drive, Madison, WI 53706 USA

[2]Department of Microbiology, University of Georgia
120 Cedar St, Athens, GA 30602 USA

Running title: lysine acetylation control of AacS in *Streptomyces*

Table S1. Strains and plasmids used in this study.

Strain or Plasmid	Relevant Genotype	Reference
E. coli strains		
MG1655	Wild type	
HB101	F$^-$, *thi-1*, *hsdS20* (rB$^-$, mB$^-$), *supE44*, *recA13*, *ara-14*, *leuB6*, *proA2*, *lacY1*, *galK2*, *rpsL20* (*str^r*), *xyl*-5, *mtl-1*	Promega
Stellar	F$^-$, *endA1*, *supE44*, *thi-1*, *recA1*, *relA1*, *gyrA96*, *phoA*, *Φ80d lacZ* Δ*M15*, Δ(*lacZYA - argF*) *U169,* Δ*(mrr – hsdRMS - mcrBC),* Δ*mcrA,* λ$^-$	Clonetech
JE6090	C41λ(DE3)	Lab Collection
JE9314	C41λ(DE3) *pka12*::*kan*$^+$	Lab Collection
Derivatives of *E. coli* strain MG1655		
JE15976	Δ*pka* Δ*cobB* Δ*atoDA* / pBAD30 *bla*$^+$	
JE15977	Δ*pka* Δ*cobB* Δ*atoDA* / pATO1 *bla*$^+$	
JE16068	Δ*pka* Δ*cobB* Δ*atoDA* / p*Sl*AacS4 *bla*$^+$	
JE16968	Δ*pka* Δ*cobB* Δ*atoDA* / p*Sl*AacS6 pSRK-Km *bla*$^+$ *kan*$^+$	
JE16969	Δ*pka* Δ*cobB* Δ*atoDA* / p*Sl*AacS6 p*Sl*PatA9 *bla*$^+$ *kan*$^+$	
Derivatives of JE17126		
JE17146	Δ*atoDA* Δ*pka* / p*Sl*AacS6 pSRK-Km *bla*$^+$ *kan*$^+$	
JE17147	Δ*atoDA* Δ*pka* / p*Sl*AacS6 *Sl*PatA9 *bla*$^+$ *kan*$^+$	
Derivatives of HB101		
JE16095	*F*$^-$, *thi-1*, *hsdS20* (rB$^-$, mB$^-$), *supE44*, *recA13*, *ara-14*, *leuB6*, *proA2*, *lacY1*, *galK2*, *rpsL20* (*str^r*), *xyl-5*, *mtl-1* / pRK2013 *kan*$^+$	(Figurski & Helinski, 1979)
Derivatives of Stellar		
JE16376	*F*$^-$, *endA1*, *supE44*, *thi-1*, *recA1*, *relA1*, *gyrA96*, *phoA*, *Φ80d lacZ* Δ*M15*, Δ(*lacZYA – argF*) *U169*, Δ(*mrr – hsdRMS –mcrBC*), Δ*mcrA*, λ$^-$ / pKC1139-Δ*patA apr*$^+$	
S. lividans strains		
TK24	Wild type	
Derivatives of *S. lividans* TK24		
JE16707	Δ*patA*	
JE16752	ΔEFD65580 ΔEFD68590 ΔEFD71509	
JE16731	TK24 / p*Sl*AacS5 *tsr*$^+$	

JE16758	Δ*aacS* (EFD70521)	
Derivative of JE16707		
JE16821	Δ*patA* / p*Sl*AacS5 *tsr*$^+$	
Plasmids		
pBAD30	P_{araBAD} expression vector, *bla*$^+$	
pTEV5	N-terminal, rTEV-cleavable His_6-tag overexpression vector, *bla*$^+$	(Rocco *et al.*, 2008)
pSRK-Km	*lacI*^{q}-*lac* promoter-operator expression vector, *kan*$^+$	(Khan *et al.*, 2008)
pSE34	P_{ermE} constitutive expression vector, *bla*$^+$ *tsr*$^+$	(Yoon *et al.*, 2002)
pRK2013	self-transmissible helper plasmid, *mob*$^+$ *tra*$^+$ *kan*$^+$	(Figurski & Helinski, 1979)
pKC1139	Temperature-sensitive shuttle vector, *apr*$^+$	(Bierman *et al.*, 1992)
pACS10	*S. enterica acs*$^+$ allele in pTYB1	(Starai *et al.*, 2002)
pATO1	*E. coli atoDA* allele in pBAD30, *bla*$^+$	
p*Sl*Acs1	*S. lividans acs*$^+$ allele (EFD68454) in pTEV5, *bla*$^+$	
p*Sl*AacS1	*S. lividans aacS*$^+$ allele (EFD70521) in pTEV5, *bla*$^+$	
p*Sl*AacS4	*S. lividans aacS*$^+$ allele (EFD70521) in pBAD30, *bla*$^+$	
p*Sl*AacS5	*S. lividans aacS*$^+$ allele (EFD70521) in pSE34, *bla*$^+$ *tsr*$^+$	
p*Sl*AacS6	*E. coli* codon-optimized *S. lividans aacS* allele (EFD70521) in pBAD30, *bla*$^+$	
p*Sl*PatA1	*S. lividans patA*$^+$ allele (EFD66247) in pTEV5, *bla*$^+$	
p*Sl*PatA9	*S. lividans patA*$^+$ allele (EFD66247) in pSRK-Km, *bla*$^+$	
pKC1139-Δ*patA*	deletion construct of *S. lividans* *patA*$^+$ allele (EFD66247) in pKC1139, *apr*$^+$	

*Unless otherwise indicated, all strains and plasmids were constructed during the course of this work.

Table S2. Primers used in this study

Name	Sequence
atoDA deletion primers	
atoDA Wanner 5’	ACCCACAACGGTGTATGCAAGAGGGATAAAAAATGAAAACGTG TAGGCTGGAGCTGCTTC
atoDA Wanner 3’	CGCGATATGCGACCAATCATAAATCACCCCGTTGCGTATTCAT ATGAATATCCTCCTTAG
Cloning primers	
atoDA+ EcoRI 5’	GCA**GAATTC**ACGGTGTATGCAAGAGGGAT
atoDA + KpnI 3’	GCC**GGTACC**CCGGCTGACAAAACGCGTC
SlAcs pTEV 5 5’	GTA**GCTAGC**ATGAGCAACGAATCCTTGGCCAAC
SlAcs pTEV5 3’	ACT**GAATTC**GCCTGTGGCCGGCTCAGTC
SlAacS pTEV5 3’	TCG**GAATTC**GCTGCTCAGGAGCGCTTG
SlAacS pBAD30 5’	GGA**GAATTC**AGGAGGACAGCTATGTCGACCGAGAACCCAC
SlAacS pBAD30 3’	TCG**AAGCTT**GCTGCTCAGGAGCGCTTG
SlAacS (opt) 5’	GGA**GAATTC**AGGAGGACAGCTATGTCGACCGAAAACCCGC
SlAacS (opt) 3’	ACT**GGTACC**TCAAGAGCGTTTGCGTGCC
SlAacS 5’ XbaI pSE34	GCC**TCTAGA**CCGGGAGCCGCCCCATGCACCACCACCACCAC CACATGTCGACCGAGAACCCAC
SlAacS 3’ HindIII pSE34	TCG**AAGCTT**GCTGCTCAGGAGCGCTTG
PatA 5’ pTEV5 NheI	GTA**GCTAGC**ATGTCGTACGCGAGCCGTACTCTGG
PatA 3’ pTEV5 EcorI	ACT**GAATTC**GGTCAGTAGGCCGGCAGGGTC
PatA 5’ pSRK-Km NdeI	GCT**CATATG**TCGTACGCGAGCCGTACTC
PatA 3’ pSRK-Km KpnI	ACT**GGTACC**TCAGTAGGCCGGCAGGGTC
SlREVPat 5’ EcoRV IF	CTATGACATGATTACGAATTC**GATATC**GGCCTAGAGTCGCTGA AAGC
SlREVPat R linker BamHI	CGTGGCGGGTCAGTAGGCC**GGATCC**CCTGCTCGTAGAAGCT GACC
SlREVPat F linker BamHI	GGTCAGCTTCTACGAGCAGG**GGATCC**GGCCTACTGACCCGCC ACG
SlREVPat 3’ HindIII IF	TGTAAAACGACGGCCAGTGCC**AAGCTT**GTCCTCGTCGAAATC GTCCGGTG

Bold typeface indicates restriction sites

Supplemental figures

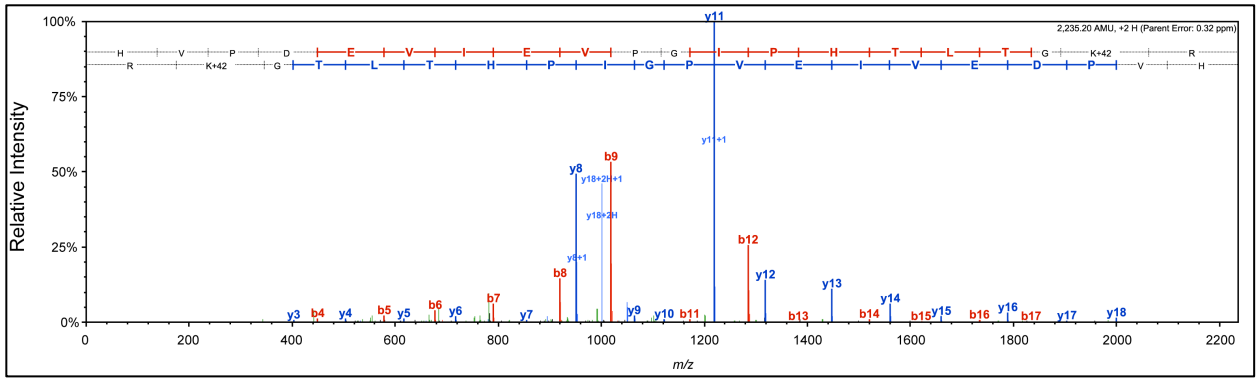

Figure S1. Residue K617 is the site of *Sl*AacS acetylation in vivo. LC/MS-MS analysis of H6-*Sl*AacS protein isolated from *S. lividans* strain JE16731 (*S. lividans* TK24 / p*Sl*AacS5) grown in NMMP medium supplemented with acetoacetate (10 mM). MS/MS spectrum of the 2235.20 amu tryptic peptide, where peaks in red represent the b series m/z (predicted fragment ion masses of HVPDEVIEVPGIPHTLTGKAcR with charge on the N-terminal amino acid), and the blue peaks indicate y series *m/z* (predicted fragment ion masses of HVPDEVIEVPGIPHTLTG**K**^{Ac}R) with the charge on the carboxy-terminal amino acid). The y (blue) series suggests the presence of a 42 atomic mass unit addition to the HVPDEVIEVPGIPHTLTGKR fragments, specifically within the final three amino acids, GKR.

Figure S2. *Sl*AacS gene cluster in Frankia sp CcI3 suggests a candidate deacetylase for *Sl*AacS. *Frankia* sp. CcI3 encodes a *Sl*PatA, *Sl*AacS, and EFD68590 homologs in a gene cluster. Percent identity (% ID) to the respective *S. lividans* homologs is reported. The figure is drawn to scale.

References

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