

Supporting information

Acetyl-CoA synthetase is acetylated on multiple lysine residues by a protein acetyltransferase with single GNAT domain in *Saccharopolyspora erythraea*

Supplemental figures

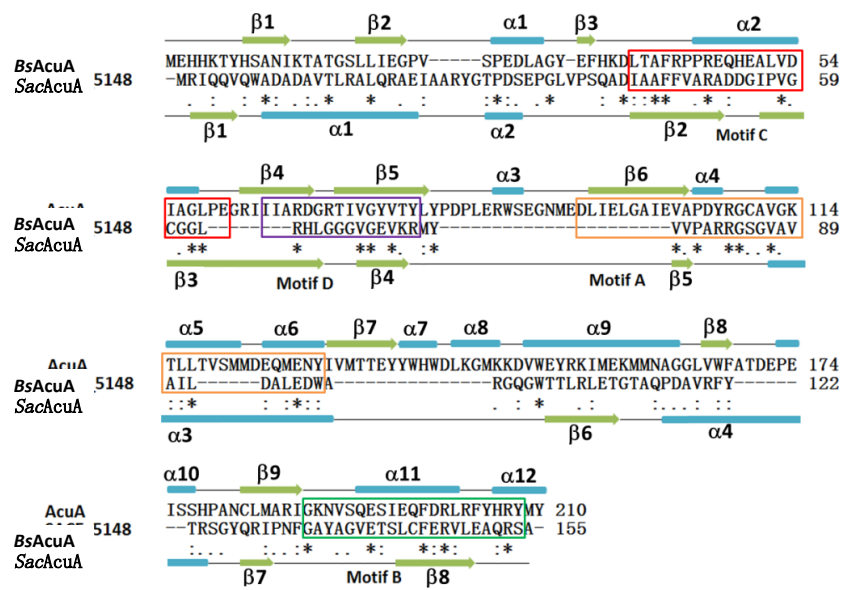


Figure S1. Sequence comparison between *BsAcuA* and *SacAcuA*. The conserved motifs, sequentially labeled C, D, A, and B were presented with boxes.

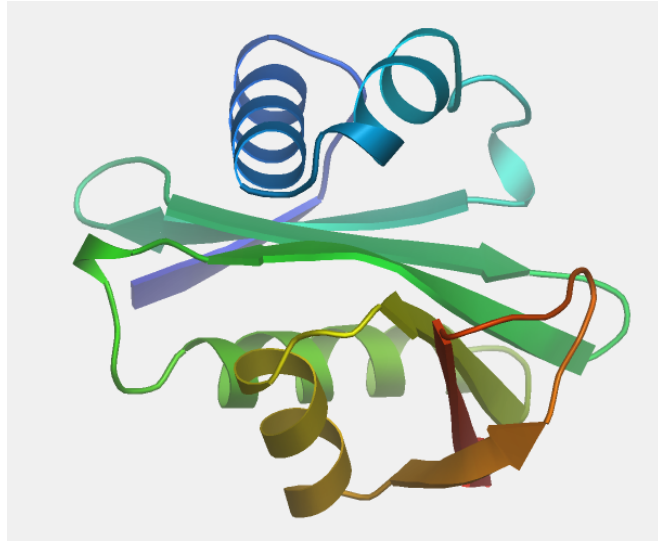


Figure S2. The structure model of SacAcuA was predicted using SWISS-MODEL Server of Protein Model Portal (PMP).

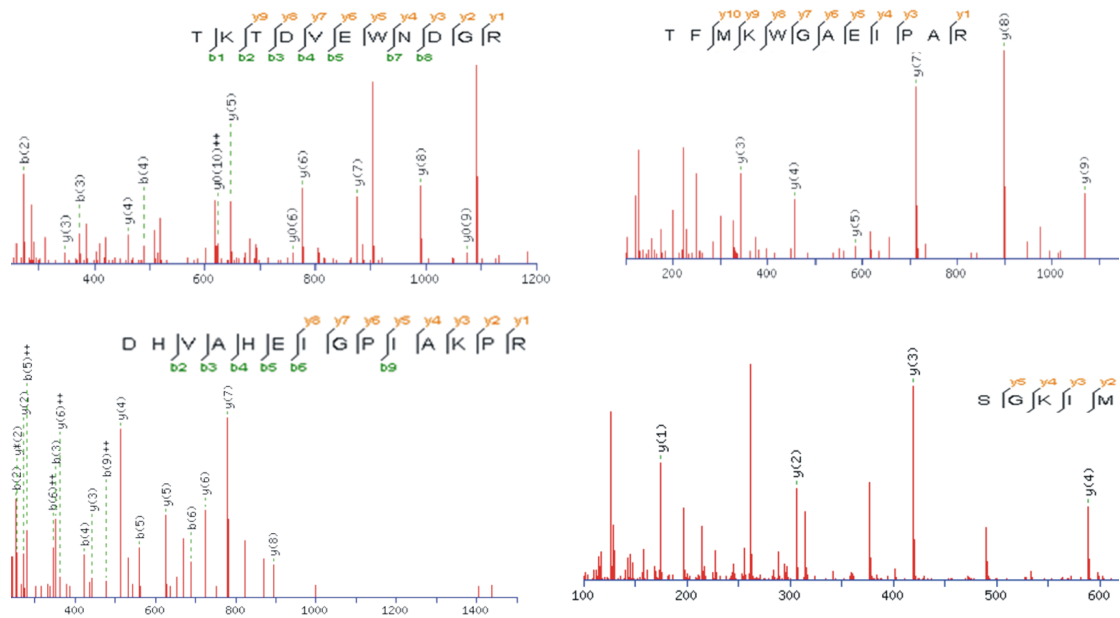


Figure S3. The MS/MS spectra of the acetylpeptides from *in vitro* acetylated SacAcuA protein by SacAcuA acetyltransferase.

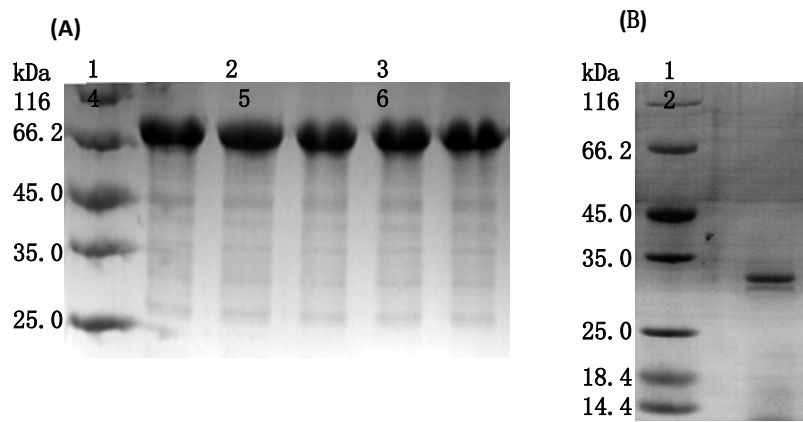


Figure. S4. SDS-PAGE analysis of purified recombinant proteins of *S. erythraea*. (A) Lane 1, the molecular mass marker; Lane 2, purified His-SacAcsA wild type; Lane 3, purified His-SacAcsA^{K237Q}; Lane 4, purified His-SacAcsA^{K380Q}; Lane 5, purified His-SacAcsA^{K611Q}; Lane 6, purified His-SacAcsA^{K628Q}. (B) Lane 1, the molecular mass marker; Lane 2, purified His-SacSrtN.

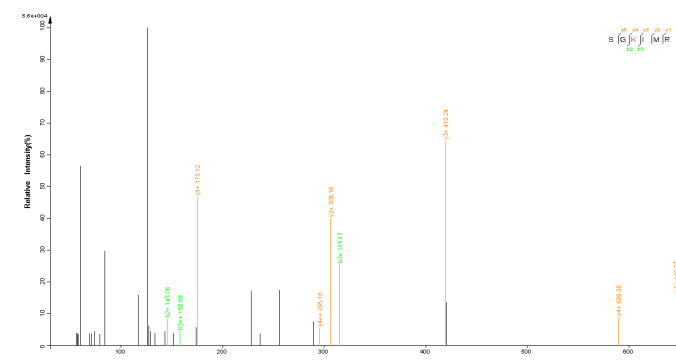
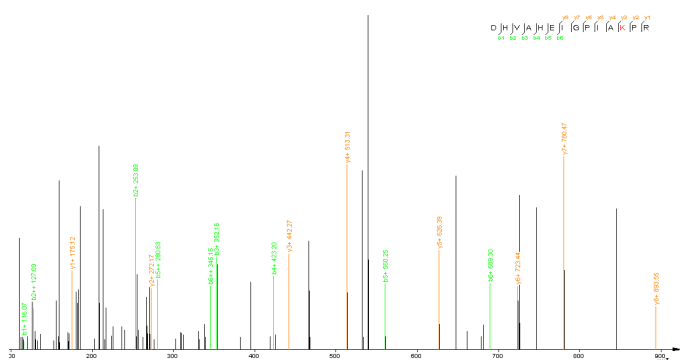
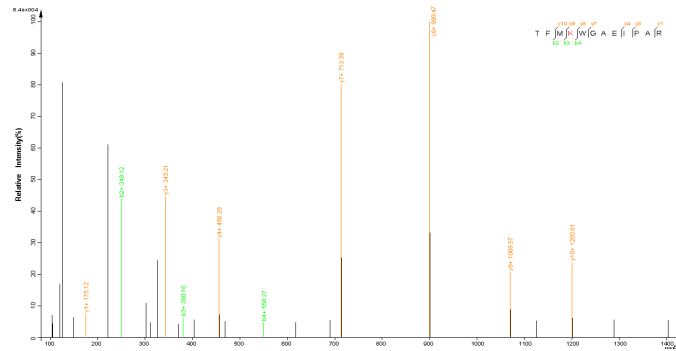
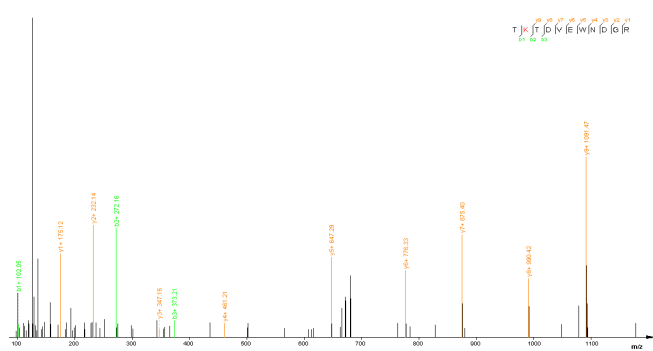


Figure S7. The MS/MS spectra of the acetylpeptides from the acetylated *SacAcS A* protein isolated from *S. erythraea* by immunoprecipitation.

Table S1. Strains and plasmids used in this work

Strain or plasmid	source or reference
strains	
<i>Salmonella enterica</i> , serovar Typhimurium LT2	ATCC 700720
<i>Bacillus subtilis</i> 168	ATCC 6051
<i>Saccharopolyspora erythraea</i> NRRL2338	DSM 40517
<i>S. erythraea</i> YE3798 (Δ SACE_3798)	In this work
<i>S. erythraea</i> YE5148 (Δ SACE_5148)	In this work
<i>Salmonella enterica</i> Δ acs2 (JE7758)	[1]
plasmids	
pET-28a	Thermo Scientific
pGEX-4T-2	Thermo Scientific
pBAD30	[2]

1. **Starai VJ, Garrity J, Escalante-Semerena JC.** 2005. Acetate excretion during growth of *Salmonella enterica* on ethanolamine requires phosphotransacetylase (EutD) activity, and acetate recapture requires acetyl-CoA synthetase (Acs) and phosphotransacetylase (Pta) activities. *Microbiol.* **151**:3793-3801.
2. **Guzman LM, Belin D, Carson MJ, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol.* **177**:4121-4130, 4312.

Table S2. Primers for overproduction of proteins

gene	Primer sequence (5'-3')
sace_2375	TAAGAATTCATGTCGTCAAACGCGTCGAGA TATAAGCTTTCAGTCGTGCTGGAGGTGG
sace_3798	TAAGAATTCTTGTTGCGCGCGGCC TATAAGCTTTCAGGTCCATGTGCTGGGGTC
sace_5148	TAAGAATTCATGAGGATCCAGCAGGTGCAGT TATGTCGACTCAGGCGCTGCGTTGTGC
Bsu29680	TAAGGATCCATGAACTTGAAAGCGTTACCAGCAA TAAGAATTCTTAATCCTCCATTGTTGACAGATCTCCA
Bsu29690	TAAGGATCCGTGGAACATCATAAAACATAACCATTGAGC TAAGAATTCTTAATACATATAACGATGATAAAAACGGAGCCT
STM4275	TAAGGATCCATGAGCCAAACACATAAACACGCCA TAAGAATTCTTATGACGGCATCGCGATGGC
STM2651	TAAGGATCCATGAGCCAGCAAGGACTGGAAG TAACCGGGTCACGATTCATCACATTTGGCCA

Table S3. Primers for constructing acetylated-site mutants of SacAcs.

Oligonucleotides	Sequences (5'-3')
K237QA	CGTCCAACGCACCCAGACCGACGTGGA
K237QB	TCCACGTCGGTCTGGGTGCGTTGGACG
K380QA	CCATCCGCACCTTCATGCAATGGGGCGCGG
K380QB	CCGCGCCCCATTGCATGAAGGTGCGGATGG
K611QA	GCCCCATCGCCCAACCCCGCCAG
K611QB	CTGGCGGGGTTGGGCGATGGGGC
K628QA	CAAACCCGCTCCGGCCAGATCATGCG
K628QB	GGCCGGAGCGGGTTTTGGGCAGTTC