Supplementary Material:

A meet-up of acetyl phosphate and c-di-GMP modulates BldD activity for development and antibiotic production

Yu Fu[1], Yu-Qi Dong[1], Jin-Long Shen[1], Bin-Cheng Yin[1], Bang-Ce Ye[1,2*], Di You[1*]

[1]Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China;

[2]Institute of Engineering Biology and Health, Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, China.

[*] Corresponding author

Corresponding authors

Bang-Ce Ye

Professor, Lab of Biosystems and Microanalysis,

State Key Laboratory of Bioreactor Engineering,

East China University of Science and Technology, Shanghai 200237, China

Tel/Fax: 0086-21-64252094

Email: bcye@ecust.edu.cn

Di You

Associate Professor, Lab of Biosystems and Microanalysis,

State Key Laboratory of Bioreactor Engineering,

East China University of Science and Technology, Shanghai 200237, China

Tel/Fax: 0086-21-64253832

Email: 030111115@mail.ecust.edu.cn

Table S1 Secondary structural elements in BldD

Protein	Helix (%)	Antiparallel (%)	Parallel (%)	β-turn (%)	Random coil (%)	Total sum (%)
BldD	44.3	6.1	6.5	15.2	25.9	98.0
$BldD^{AcuA}$	61.8	3.5	4.0	12.7	17.6	99.5
$BldD^{AcP}$	82.0	1.5	1.8	9.8	8.9	104.0

Table S2 Strains and plasmids used in this work

Strain or plasmid	Characteristics	Source or Reference
Strains		
S. erythraea NRRL2338	Used as parental strain, wild type	DSM 40517
E. coli DH5α	*E. coli* DH5α F-Ø80d lacZΔM (lacZ YA -argF) U169 deoR	Transgen Biotech
E. coli BL21(DE3)	F'ompTr- Bm-B (DE3)	Transgen Biotech
E. coli BL21(DE3)- *bldD*	The strain for expression of BldD	This study
E. coli BL21(DE3)- $bldD^{K11Q}$	The strain for expression of $BldD^{K11Q}$	This study
E. coli BL21(DE3)- $bldD^{K11R}$	The strain for expression of $BldD^{K11R}$	This study
S. erythraea Ob*ldD*	The strain for over-expression of *bldD*, NRRL2338 integrated with pIB139-*bldD*	This study
S. erythraea ObldD^{K11Q}	The strain for over-expression of $bldD^{K11Q}$, NRRL2338 integrated with pIB139-$bldD^{K11Q}$	This study
S. erythraea ObldD^{K11R}	The strain for over-expression of $bldD^{K11R}$, NRRL2338 integrated with pIB139-$bldD^{K11R}$	This study
plasmid		
pET28a(+)	vector with T7-RNA polymerase-based promoter for expression in *E. coli* BL21(DE3), hexahistidine tag with thrombin cleavage	Thermo Scientific
pET- *bldD*	pET28a(+) with *bldD* inserted in NdeI and HindIII	This study
pET- $bldD^{K11Q}$	pET28a(+) with $bldD^{K11Q}$	This study
pET- $bldD^{K11R}$	pET28a(+) with $bldD^{K11R}$	This study
pIB139	pSET152 with integrase of phiC31 and PermE, the strong promoter of *Streptomyces*	(25)
pIB139- *bldD*	pIB139 with *bldD* gene interted intoNdeI and XbaI	This study
pIB139- $bldD^{K11Q}$	pIB139 with $bldD^{K11Q}$	This study
pIB139- $bldD^{K11R}$	pIB139 with $bldD^{K11R}$	This study

Table S3 The oligonucleotides used in the study

Oligonucleotides	Sequence (5'to 3')
Primers for overproduction of BldD protein	
pET-*bldD*F	GTCGCGGATCCGAATTCATGGGCGACTACGCCAAG
pET-*bldD*R	GGTGCTCGAGTGCGGCCGCAAGCTTTCACTCCTCCCGGGCCG
Primers for the construction of the *S. erythraea* Ob*ldD* strain	
pIB-*bldD*F	GCCGGTTGGTAGGATCCACATATGCATCATCATCATCATCACAGCAGCG
pIB-*bldD*R	CGCGCGCGGCCGCGGATCCTCTAGAAGCTTCCTTTCGGGCTTTGTTAG
M13F	GTGCTGCAAGGCGATTAAGTT
M13R	TTATGCTTCCGGCTCGTATGT
The primers used in Site-Directed Mutagenesis of BldD	
K11R F	GCGGCAGGCTCCGCGC
K11R R	CTGCCGCCCAGCGCCT
K11Q F	GCGGCCAGCTCCGCGC
K11Q R	GGCCGCCCAGCGCCTT
Primers for PCR amplification of EMSAs probe with biotin labeling	
*bldD*_F	AGCCAGTGGCGATAAGTCCCGACGGCTGTCGGC
*bldD*_R	AGCCAGTGGCGATAAGCCGTTTCATTCGCCCCGTC
Primers for real-time RT-PCR	
SACE_8101-F	GTTGCGATGCCGTGAGGT
SACE_8101-R	CGGGTGTTACCGACTTTCA
eryBIV-F	GCAGCCGCAGGATCACGC
eryBIV-R	GCCGCCCGTGTTGCTCTA
eryAI-F	CCGCTGATGCCGAACGAC
eryAI-R	CACCCTTCCCCGCACTCTG
ermE-F	CCTCCAGGCACCAGTCCAC
ermE-R	AGTCGTTGCGGGAGAAGCT
eryK-F	CCGATGGACCACGAGCAGTT
eryK-R	AAGGCGGGAGATCAGGTCGT
whiG-F	AGTTGCAGATGACCAGCGTG
whiG-R	GAGACGTTCTACAGCCTCGG
bldN-F	CTCTACGACGAGTACTCCCAGG
bldN-R	GTTCTTGGCGATGGTGATGAAC
bldM-F	GACTTGTCTCGGCCCACC
bldM-R	CTCGGTCAGCTGCACACC

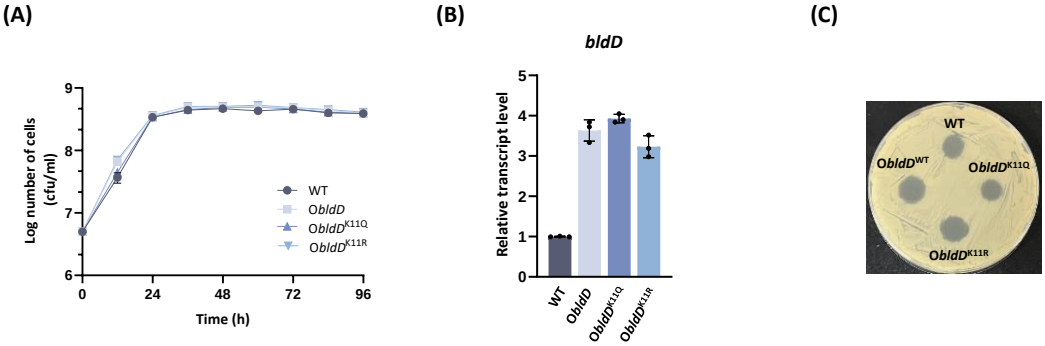

Figure S1 K11 acetylation inhibits antibiotic production. (A) Growth curves of *S. erythraea* WT, O*bldD*, O*bldD*^{K11Q}, and O*bldD*^{K11R} strains grown in TSB medium. (B) The transcription level of *bldD* gene in *S. erythraea* WT, O*bldD*, O*bldD*^{K11Q}, and O*bldD*^{K11R} strains grown in TSB medium till the middle exponential phase. Fold change represented the expression level compared to WT strain. Error bars show the SDs of three independent experiments. (C) The antibiotic production of *S. erythraea* WT, O*bldD*, O*bldD*^{K11Q}, and O*bldD*^{K11R} strains grown in TSB medium determined by bioassays.

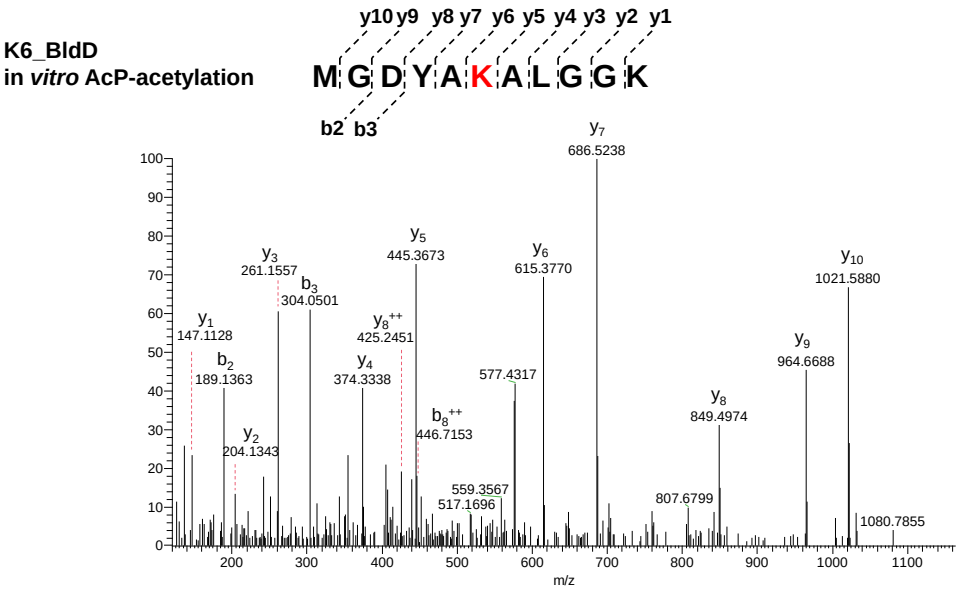

Figure S2 MS/MS spectra for the identification of AcP- acetylated K6 by LC/MS/MS analysis.

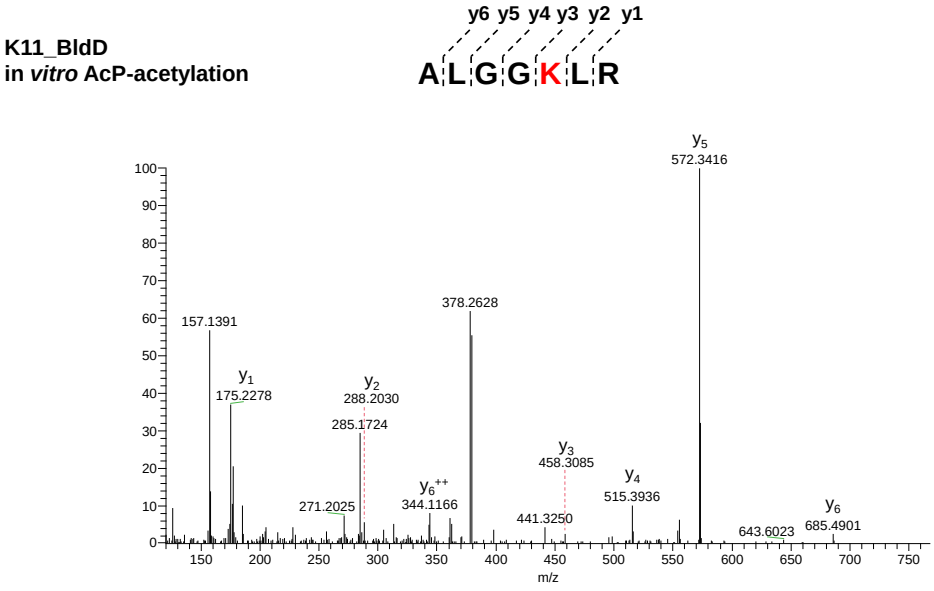

Figure S3 MS/MS spectra for the identification of AcP- acetylated K11 by LC/MS/MS analysis.

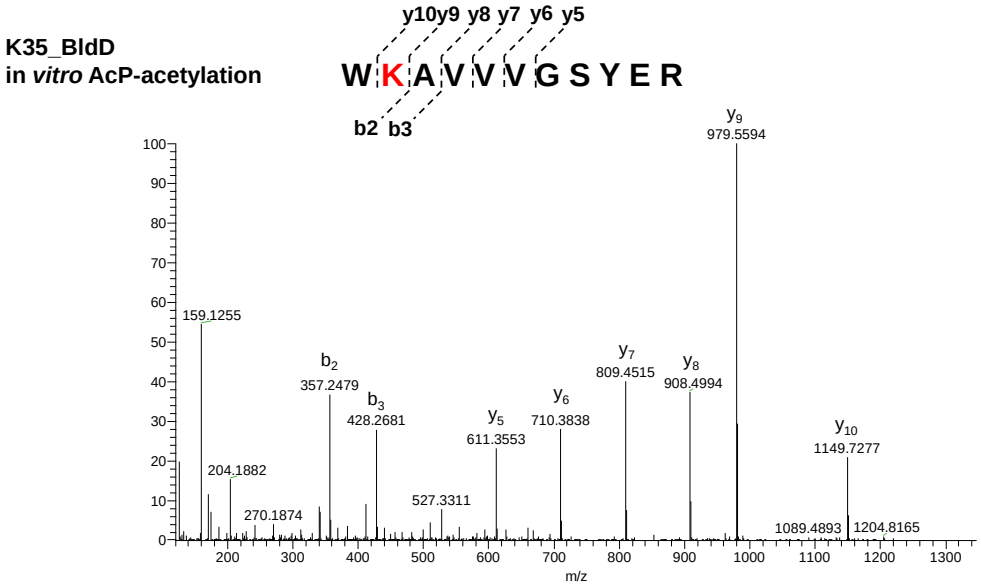

Figure S4 MS/MS spectra for the identification of AcP- acetylated K35 by LC/MS/MS analysis.

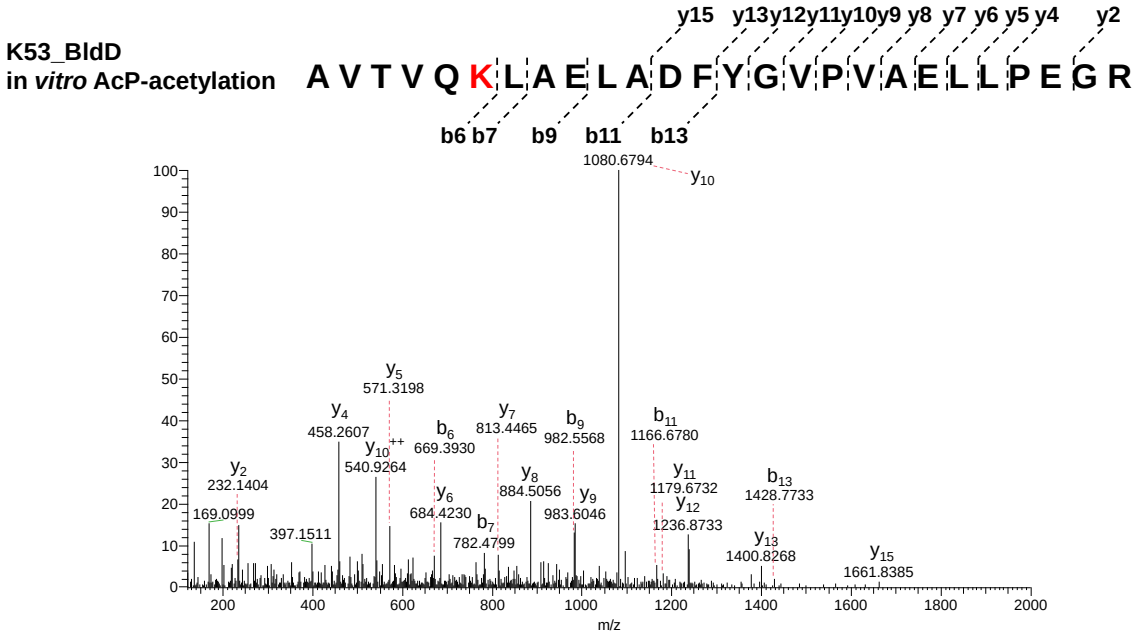

Figure S5 MS/MS spectra for the identification of AcP- acetylated K53 by LC/MS/MS analysis.

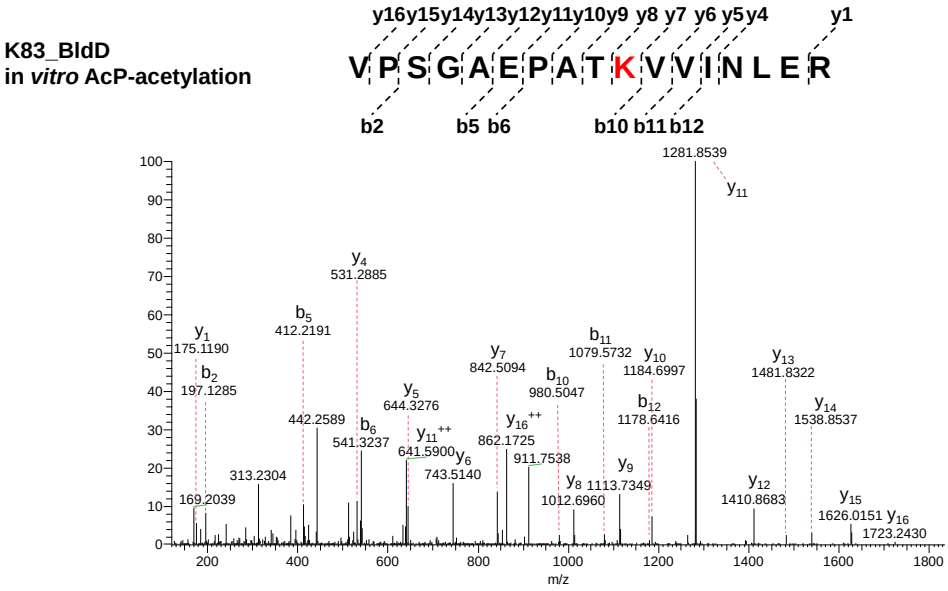

Figure S6 **MS/MS spectra for the identification of AcP- acetylated K83 by LC/MS/MS analysis.**

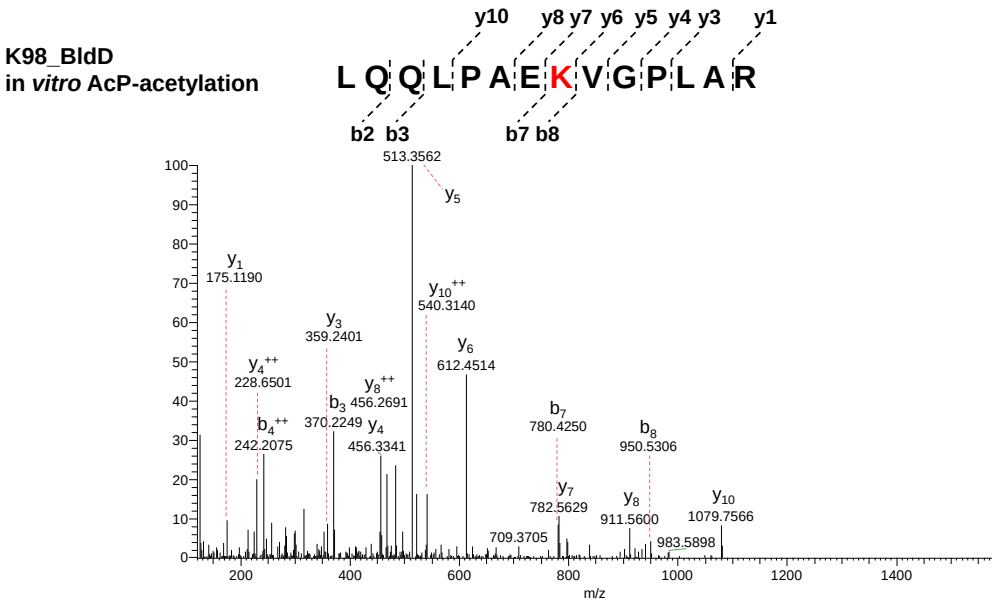

Figure S7 **MS/MS spectra for the identification of AcP- acetylated K98 by LC/MS/MS analysis.**

K119_BldD
in *vitro* AcP-acetylation

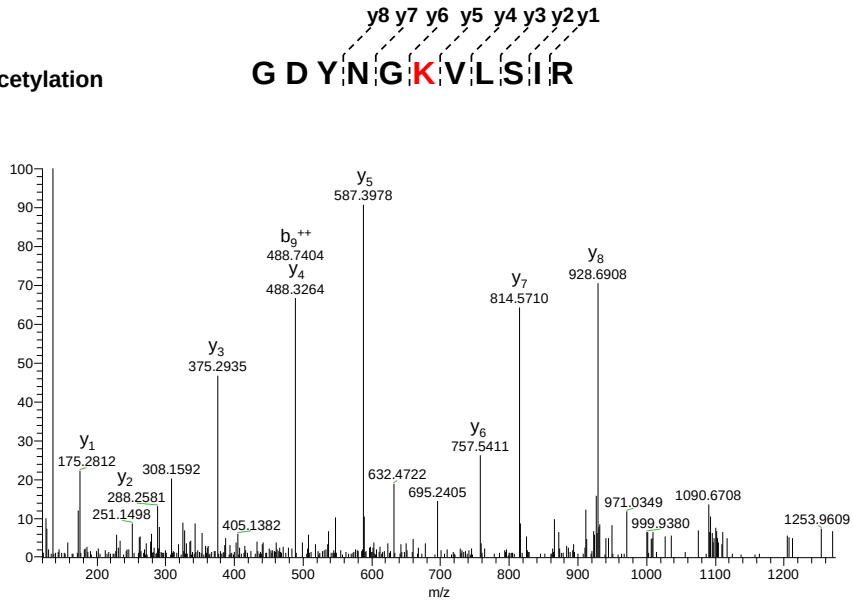

Figure S8 MS/MS spectra for the identification of AcP- acetylated K119 by LC/MS/MS analysis.

K35_BldD
in *vitro* AcuA-acetylation

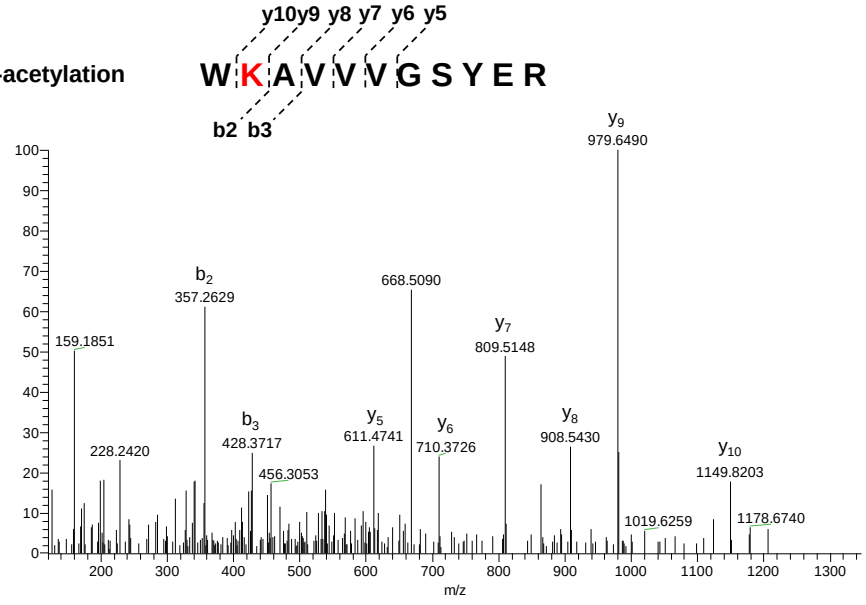

Figure S9 MS/MS spectra for the identification of AcuA- acetylated K35 by LC/MS/MS analysis.

K53_BldD
in *vitro* AcuA-acetylation

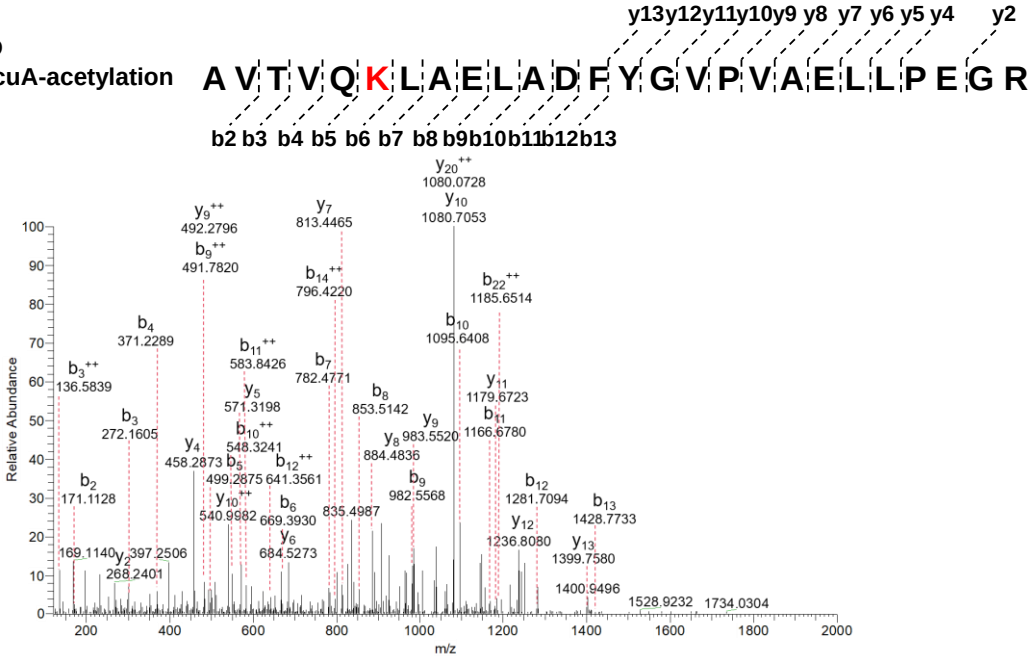

Figure S10 **MS/MS spectra for the identification of AcuA- acetylated K53 by LC/MS/MS analysis.**

K83_BldD
in *vitro* AcuA-acetylation

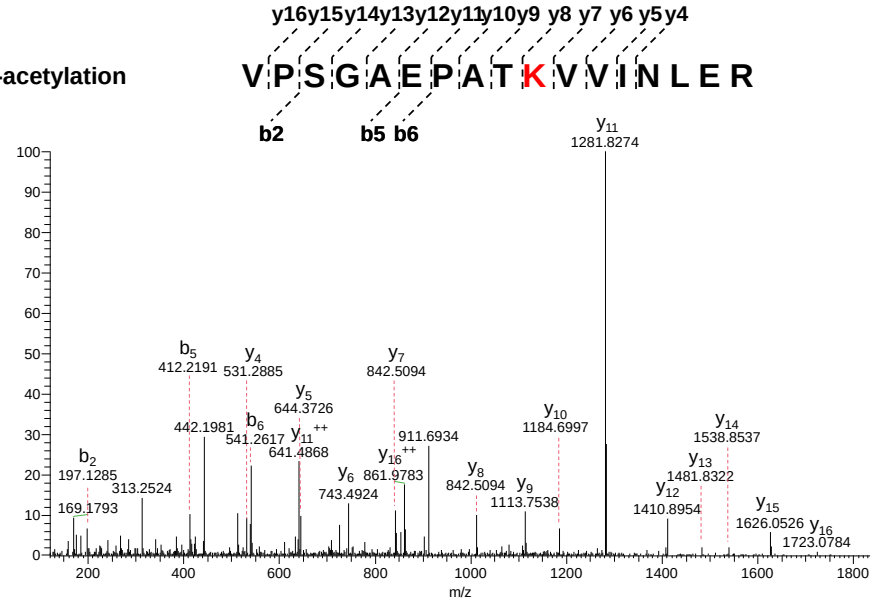

Figure S11 **MS/MS spectra for the identification of AcuA- acetylated K83 by LC/MS/MS analysis.**

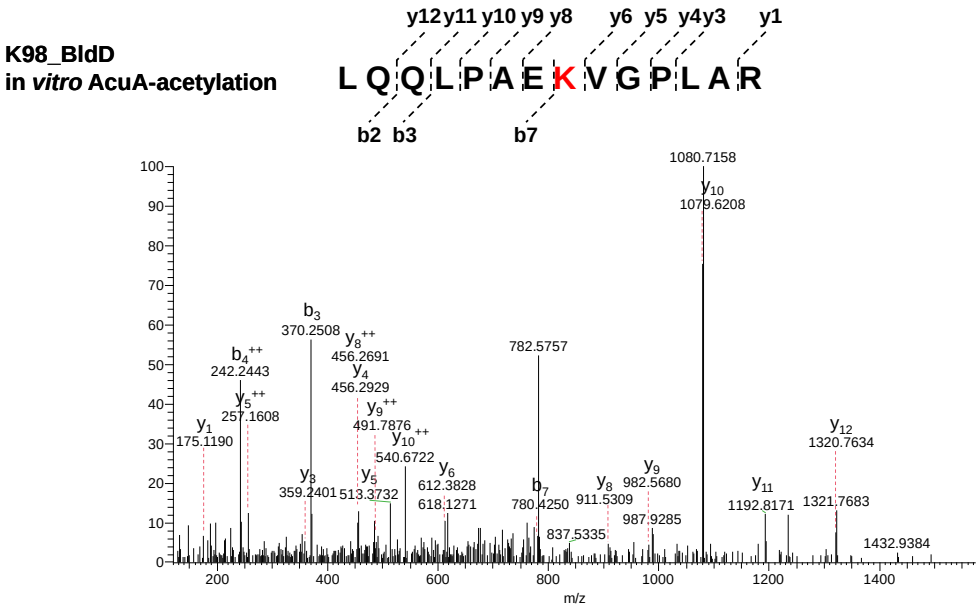

Figure S12 MS/MS spectra for the identification of AcuA- acetylated K98 by LC/MS/MS analysis.

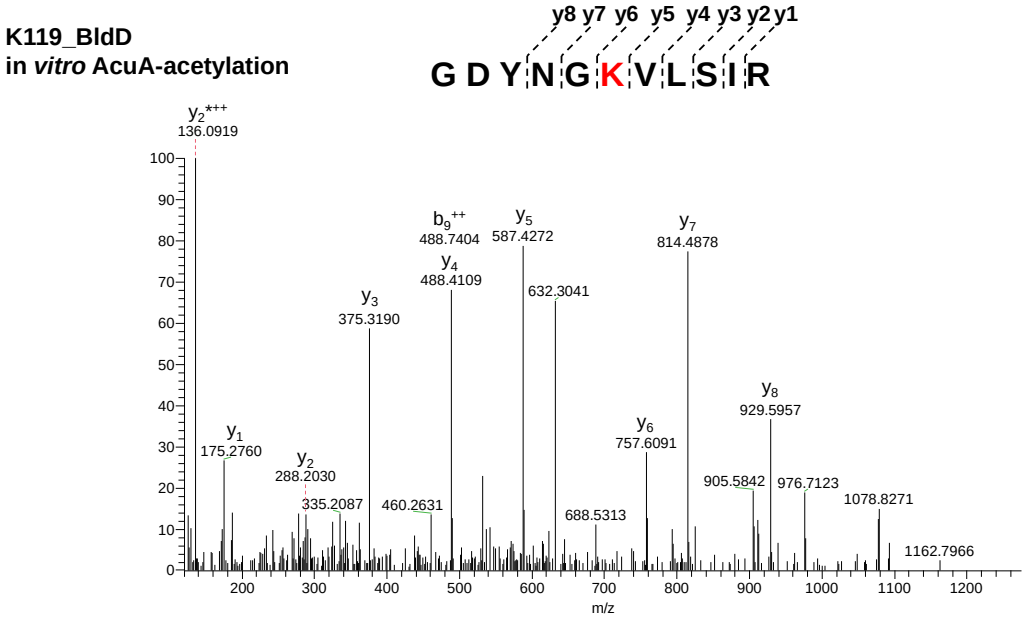

Figure S13 MS/MS spectra for the identification of AcuA- acetylated K119 by LC/MS/MS analysis.

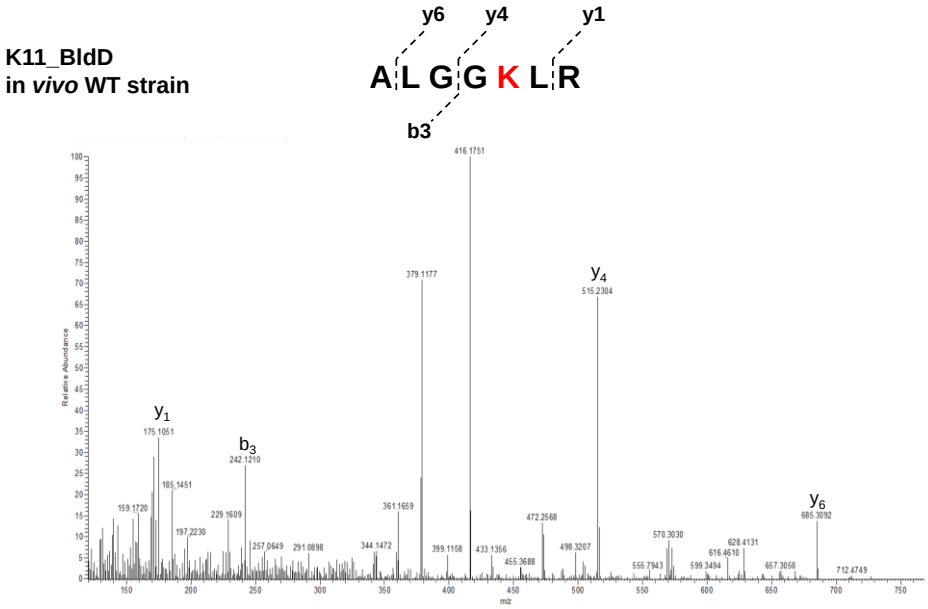

Figure S14 MS/MS spectra for the identification of K11 *in vivo* by LC/MS/MS analysis.

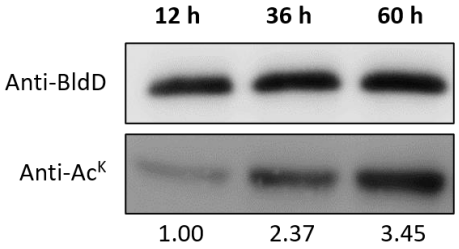

Figure S15 BldD acetylation level at different growth stage. Acetylation level of BldD of the *S. erythraea* WT strain under N^- conditions at the indicated time. Each lane was loaded with equal amount of BldD protein. The band intensities were quantified by densitometry using ImageJ software.