

# **Enhanced bacitracin production by systematically engineering S-adenosylmethionine supply modules in *Bacillus licheniformis***

Running title: Enhancing bacitracin production in *B. licheniformis*

Dongbo Cai <sup>a</sup>, Bowen Zhang <sup>a</sup>, Jiang Zhu <sup>a</sup>, Haixia Xu <sup>a</sup>, Pei Liu <sup>a</sup>, Zhi Wang <sup>b</sup>, Junhui Li <sup>c</sup>,  
Zhifan Yang <sup>a\*</sup>, Xin Ma <sup>a</sup>, Shouwen Chen <sup>a\*</sup>

<sup>a</sup> *State Key Laboratory of Biocatalysis and Enzyme Engineering, Environmental Microbial Technology*

*Center of Hubei Province, College of Life Sciences, Hubei University, Wuhan, 430062, PR China*

<sup>b</sup> *Hubei Provincial Key Laboratory of Industrial Microbiology, Key Laboratory of*

*Fermentation Engineering (Ministry of Education), School of food and biological engineering,*

*Hubei University of Technology, Wuhan 430068, Hubei, China*

<sup>c</sup> *Lifecome Biochemistry Co. Ltd, Nanping, 353400, PR China*

\*Corresponding author: Prof. Zhifan Yang and Shouwen Chen

Tel./fax.: +86 027-88666081.

*E-mail address:* [sailyangzhf@hubu.edu.cn](mailto:sailyangzhf@hubu.edu.cn) and [mel212@126.com](mailto:mel212@126.com) (S. Chen).

*Postal address:* 368 Youyi Avenue, Wuchang District, Wuhan 430062, Hubei, PR China

**Table S1** The primers used in this research

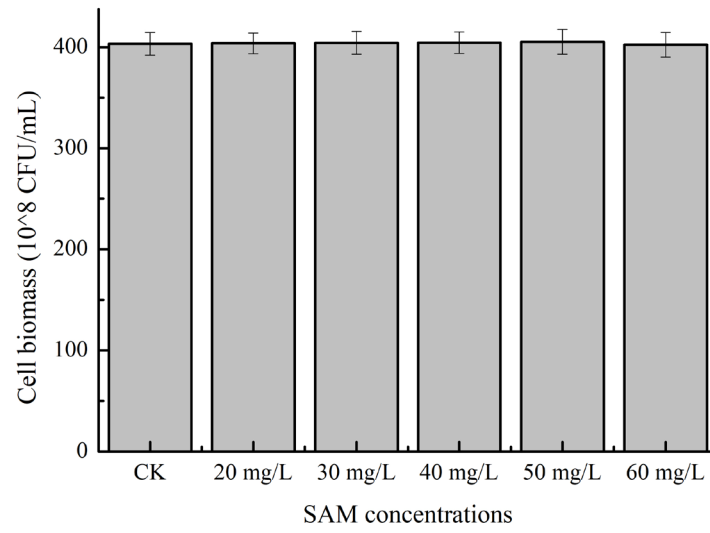
Primer names	Sequences (from 3' to 5')
pHY-F	GTTTATTATCCATACCCTTAC
pHY-R	CAGATTTCGTGATGCTTGTC
T2-F	ATGTGATAACTCGGCGTA
T2-R	GCAAGCAGCAGATTACGC
P43-F	CGGAATTC TGATAGGTGGTATGTTTTTCG
P43-R	GTGTACATTCCTCTCTTACCTA
SAM2-F	TAAGAGAGGAATGTACACATGTCCAAGAGCAAACTTTCT
SAM2-R	TCCGTCCTCTCTGCTCTTTTAAAATTCCAATTTCTTTGGT
MetK <sub>Bl</sub> -F	TAAGAGAGGAATGTACACATGAGCAAAAACCGTCGGTTA
MetK <sub>Bl</sub> -R	TCCGTCCTCTCTGCTCTTTTAAATTATTCTCCTAATGCATCTTTC
MetK <sub>Cg</sub> -F	TAAGAGAGGAATGTACAC
MetK <sub>Cg</sub> -R	TCCGTCCTCTCTGCTCTTT
TamyL-F	AAGAGCAGAGAGGACGGATTC
TamyL-R	GCTCTAGAGCCGCAATAATGCCGTCGCACTG
SAM2-F1	CGGGATCCCGTGAGGCGATGGATGTTCT
SAM2-R1	CGAAAACATACCACCTATCATTGACGAACCGTATCCGC
SAM2-F2	GCGGATACGGTTCGTCAATGATAGGTGGTATGTTTTTCG
SAM2-R2	TCTTACCGTTTGCTGAGTGCGCAATAATGCCGTCGCACTGGC
SAM2-F3	GCCAGTGCGACGGCATTATTGCGCACTCAGCAAACGGTAAGA
SAM2-R3	GCTCTAGAGCTGTCAAACGCTCCGGTGG
SAM2-KYF	AGCTTCAATGCTACCCAAGCAGC
SAM2-KYR	GCCTTGTCTGAAATACATATA
MetH <sub>Bl</sub> -F	TAAGAGAGGAATGTACACATGACAAATGTAAAAACGAG
MetH <sub>Bl</sub> -R	TCCGTCCTCTCTGCTCTTTTAAATTAACAGTCTGAGCGAGTTTG
MetH <sub>Cg</sub> -F	TAAGAGAGGAATGTACACATGAGCCAGAACCGCATCAGGACC
MetH <sub>Cg</sub> -R	TCCGTCCTCTCTGCTCTTTTATAGAACAGTTCCTTTGATGCAA
Met6-F	TAAGAGAGGAATGTACACATGGTTCAATCTGCTGTCTTA
Met6-R	TCCGTCCTCTCTGCTCTTTTAAATCTTGTATTGTTACGG
PmetH-F1	CGACTTCCGGGAGCGACTTC
PmetH-R1	TGAATCTCGCCGAAATCGCAGGTTGTTTTCTCCTTTCTTTC
PmetH-F2	GAAAGAAAAGGAGAAAACAACCTGCGATTTCGGCGAGATTCA
PmetH-R2	CAGCTGGTCATTGATGTTAGACATATAAAAATTCTCCTTTTTGAT
PmetH-F3	ATCAAAAAGGAGAATTTTTATATGTCTAACATCAATGACCAGCTG
PmetH-R3	CCCATCGGTTTCGATCGTGCCCTGA
PmetH-KYF	ATATGAGGTCGCTGATCCATATT
PmetH-KYR	CTGACGGCCGTCCTCGCAAGGGAA
metN-F1	GCTCTAGACACGAATCTGATTGACAA
metN-R1	TCTGTAATCGTTCGGTGCCCATGCCGATCTTCCTTT
metN-F2	AAAGGAAGATCGGCATGGGCACGGAACGATTACAGA
metN-R2	GCGAGCTCAAACCTCAGCACAAGCGAACC
metN-KYF	TTAAACGAAATTACAACCTGCTA
metN-KYR	TAGCCGTATGTGATCGCC

---

metP-F1	GCTCTAGACGGAGGTTCTGCTCTGTG
metP-R1	AACTCAGCACAAGCGAACGACGAGCCTTGCGATGTA
metP-F2	TACATCGCAAGGCTCGTCGTTGCTGTGCTGAGTT
metP-R2	GCGAGCTCTTGGCGTCAATGTCACCG
metP-KYF	CGGAGGTTCTGCTCTGTG
metP-KYR	ACCGCTATGCCGTTGTTG
metN-F	TAAGAGAGGAATGTACACATGATTACATTTGAAGGCGTTGAA
metN-R	TCCGTCCTCTCTGCTCTTTTAATTATACCTCCCTGATGCGAATG
metP-F	TAAGAGAGGAATGTACACATGTTTGA AAAACTGTTTCCAA
metP-R	TCCGTCCTCTCTGCTCTTTTACTCAGAAACAGAAATGACA
PmetPQ-F1	GCTCTAGATCAGGCACGGTTGAAGTA
PmetPQ-R1	TCTCGCCGAAATCGCAGGATGCTTCAGCGTTTCCTGT
PmetPQ-F2	ACAGGAAACGCTGAAGCATCCTGCGATTTCCGGCAGA
PmetPQ-R2	CTTCCACAGCTGCTCCATATAAAAATTCTCCTTTTTGAT
PmetPQ-F3	ATCAAAAAGGAGAATTTTTATATGGAGCAGCTGTGGGAAG
PmetPQ-R3	GCGAGCTCGCCGTTTACATAGGCAAGG
PmetPQ-KYF	AGTGCCCTATGAAACCCG
PmetPQ-KYR	AGCGGCTTTGCTTCTTCA
speD-F1	GCTCTAGAGTTGACCTTGTTGGCTGAT
speD-R1	AATCGGCAGCTACGTTCCGATCCCCACAGTTCGGAGATA
speD-F2	TATCTCCGAACTGTGGGGGATCCGAACGTAGCTGCCGAT
speD-R2	GCGAGCTCCTTCCAGCACTTCCTTTAC
speD-KYF	GGAAGAATGCACGGCCTGGC
speD-KYR	CTCCTCTTTCTTCCGACACC
mtnN-F1	GCTCTAGACGGAAAGGGAGTCACAGG
mtnN-R1	TGATAGGAAACCTGTGCGATTGACTTTGCCGATGCC
mtnN-F2	GGCATCGGCAAAGTCAATCGCACAGGTTTCCTATCA
mtnN-R2	GCGAGCTCATCGCACCCCTTCATTCCG
mtnN-KYF	CGGGTGACAAGGGAAATA
mtnN-KYR	TTATGTCCGTGCGGTTCT
RT-bacA-F	GTATCTGCCGATCGACCCTG
RT-bacA-R	TCACAATCGATTCCGTCGCG
RT-bacT-F	ACTATCAAATGAGCGGGGCG
RT-bacT-R	GGATTGGGCAAGGTATGCAG
RT-bacB-F	GGACGAATCAGGCGAGAGAG
RT-bacB-R	GGACGAATCAGGCGAGAGAG
RT-bacC-F	GGACGAATCAGGCGAGAGAG
RT-bacC-R	TGATCTTCCCTTCGCCACCAC
RT-abrB-F	CTAGGACGCGTGGTGATTCC
RT-abrB-R	CCAATTTACCGCCTGCAAGT

---

**Fig. S1** Effects of SAM addition on the cell growth of *B. licheniformis*.



**Fig. S2** The concentrations of intracellular Met and SAM before (24 h) and after (30 h) SAM addition.

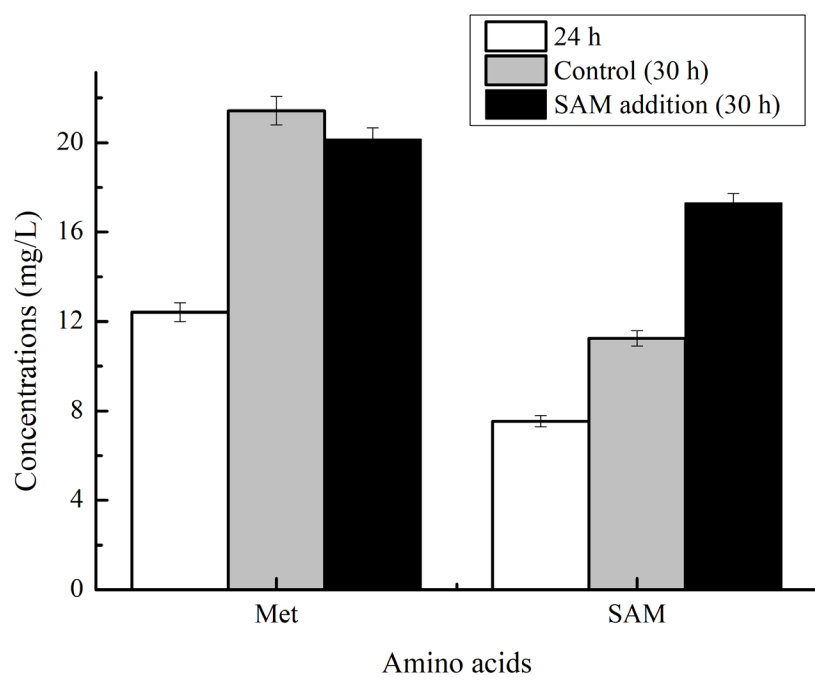


Fig.S3 Effects of SAM addition on the transcriptional level of regulator gene *abrB*.

