Electronic Supplementary Materials for

Studies on a blasticidin S deaminase from *Streptomyces lividans* and its application in engineering a blasticidin S producing strain for easy genetic manipulation

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Supplementary materials

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Table S1. Bacterial strains, plasmids and cosmids		
Strain	Relevant properties	Source or Reference
S. lividans		
66 (NRRL B-16148)	Wild type, Dnd^+, ø$HAU3^r$, $SLP2^+$, $SLP3^+$	(1)
HXY16	*S. lividans* 66 derivative with genomic island SLG precisely excised at the 15 bp *attB* site	(2)
LL1	*S. lividans* HXY16 derivative with 12kb fragment insertion containing part of the BS biosynthesis gene cluster and an *aadA* cassette at the genome	This study
LL2	*S. lividans* LL1 derivative with the whole BS biosynthesis gene cluster inserted into the genome.	This study
WJ1	*S. lividans* HXY16 derivative with *SLBSD* disruption	This study
WJ2	*S. lividans* LL2 derivative with *SLBSD* disruption	This study
WJ3	*S. lividans* WJ2 derivative with *blsL* disruption	This study
WJ4	*S. lividans* WJ2 derivative with *blsE* disruption	This study
WJ5	*S. lividans* WJ2 derivative with *blsF* disruption	This study
Streptomyces griseochromogenes	Producer of blasticidin S	China General Microbiological culture collection center (CGMCG)
Rhodotorula rubra	Indicator strain of blasticidin S	Xu Zhinan, personal communication
Escherichia coli DH10B	F^- *rec*A *lac*Z △M15	GIBCO BRL
ET12567 /pUZ8002	Strain used for conjugation between *E. coli* and *Streptomyces* spp. *rec*F, *dam*, *dcm*, *hsdS*, Cml^r, Str^r, Tet^r, Km^r	(3)
EPI300-$T1^R$	F- mcrA □ (*mrr-hsd*RMS-*mcr*BC) 80d *lac*Z M15 *lac*X74 *rec*A1 *end*A1 *ara*D139 (*ara*, *leu*)7697 *gal*U *gal*K - *rps*L *nup*G *trf*A *ton*A *dhfr*	EPICENTRE Biotechnologies
BL21(DE3)plysE	F- ompT rB- mB- (λDE3) pLysE Cm^r	Novagen
BW25113	*lacIq* rrnBT14 ΔlacZWJ16 *hsdR514* ΔaraBADAH33 Δ*rha*-BADLD78	the Coli Genetics Stock Center
Plasmids		
pJTU412	Shuttle cosmid derived from pHZ1358, *oriT*, *ori*(ColE1),	(4)

	bla, tsr, cos, rep(pIJ101), *ori*(pIJ101)	
pOJ260	*aac(3)IV, oriT, rep*^{pUC}, *lac*Z	(5)
pJTU1289	cosmid vector derived from pJTU412, *ori*(ColE1), *bla*, *tsr, cos*	(6)
pCC1FOS	Vector for genomic library	Epicentre
pIJ779	pBluescript KS(+), aadA, FRT sites	(7)
7D11	pCC1FOS derived fosmid with insertion from *S. griseochromogenes* containing 34.5kb fragment of blasticidin S biosynthetic cluster	This study
pJTU1780	A 36kb fragment from 7D11 cut by SpeI and XbaI inserted at the SpeI-XbaI site of pJTU1289	This study
pJTU1528	pOJ260 with 4.85-kb dispensable fragment of *S. lividans* HXY16 amplified by Primer ZX1 at EcoRV site	This study
pJTU1785	pJTU1780 digested with StuI and ligated with an *aadA* cassette	This study
pJTU1786	A 12kb fragment cut from pJTU1785 by EcoRV inserted at the StuI site of pJTU1528	This study
pWJ1	A 2kb fragment amplified from *S. lividans* HXY16 with primer BKO-UP inserted at the *Sma*I site of pIJ2925	This study
pWJ2	A 2kb fragment amplified from S. lividans 66 with primer BKO-DOWN inserted at the *Sma*I site of pIJ2925	This study
pWJ3	Two 2kb *Xba*I-*Kpn*I fragments from pWJ1 and pWJ2, together with a *Kpn*I-*Kpn*I kan^{r} cassette amplified from SuperCos1 with primers *Kan-Kf* inserted at *Xba*I site of pJTU412	This study
pWJ4	*SLBSD* expression vector, PCR product for *SLBSD* has NdeI and EcoRI restriction sites at its two ends, which were used for cloning into corresponding sites of pET28a(+)	This study
pWJ5	pJTU412 with insertion of a ca. 13kb BglII fragment of the sequenced blasticidin S biosynthetic cluster(from 24646-36677) at the BamHI site	This study
pWJ6	The *blsE* in the pWJ4 substituted by *aadA* using the PCR-Targeting method	This study
pWJ7	The *blsL* in the pWJ4 substituted by *aadA* using the PCR-Targeting method	This study
pWJ8	The *blsF* in the pWJ4 substituted by *aadA* using the PCR-Targeting method	This study

Table S2. Primers used in this research		
Primers	Forward (F)	Reverse (R)
aadA-T	TCGGCAGCGACATCCTTC	CGTCATCGAGCGCCATCT
ZX1	TGAGCACCGACACCGAGTT	GCACCTTCAGGCTGTAGGGA
CMPH	GACGGTCTACAACGCCCACC	GCCCAGCCGATCTCCACA
BKO-UP	TCTAGACTGGCCGCCACCGGGAGCCCATACGTCAC	GGTACCCGTCTCTGCCTTGTCAGGTGTGGTGTCAG
BKO-DOWN	GGTACCGACCGGACGTCAGAGCGCGAGTCCGGTGA	TCTAGACTCGGCACCGTCCGGGCCCATCGCCG
Con-BKO	ACCGGCCGACACCTGACACCACACCT	AGCGGGTGCTGGTCCTCACCGGACTC
KanK	CGGGGTACCCTATTCCAGAAGTAGTGAGG	CGGGGTACCCTGGATGCCGACGGATTTG
SLBSD	CGGCATATGACCTCGCAGACGAACCCCGTCGA	CGGGGATCCTCACCCGGCGTCGAGCTGGTGGTCC
Tar-blsE	AGACCGGCTGCCGCCGGCCCACAGGAAGGTTCCGCCATGATTCCGGGGATCCGTCGACC	GCCTTGACCGATGGGGACCCGGGCCGCTCGGGCGGATCATGTAGGCTGGAGCTGCTTC
Tar-blsF	GCCCGGGTCCCCATCGGTCAAGGCGACAGGAGAGAGATGATTCCGGGGATCCGTCGACC	GGCTGTCCCGCGGACAGCGTGGCGGATGAGTGGGATTCATGTAGGCTGGAGCTGCTTC
Tar-blsL	GGCGGCCAAGACGCTCCGGCAGACACTGGAGTCGCTGTGATTCCGGGGATCCGTCGACC	GTCGTGGACGGGTCGGGCGCGCGGGCGGCGCGGCTCATGTAGGCTGGAGCTGCTTC
Con-blsE	TACGGACCCGGGCCGACATACGTGA	GCCGGCCCGTGCGCCGGGAACGCTT
Con-blsF	GGCGTACGGCCGTGGCAGTACTGAT	CGGGTCGGAAAAACACCTTGCTGGGT
Con-blsL	ACCACCAGGGACGTGACGTTCCGGGT	TCCTCCGGTGTCAGCACCTGCGCA

Reference

1. **Zhou, X., X. He, A. Li, F. Lei, T. Kieser, and Z. Deng.** 2004. *Streptomyces coelicolor* A3(2) lacks a genomic island present in the chromosome of *Streptomyces lividans* 66. Appl Environ Microbiol **70:**7110-7118.

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3. **Paget, M. S., L. Chamberlin, A. Atrih, S. J. Foster, and M. J. Buttner.** 1999. Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J Bacteriol **181:**204-211.

4. **Sun, Y., X. He, J. Liang, X. Zhou, and Z. Deng.** 2009. Analysis of functions in plasmid pHZ1358 influencing its genetic and structural stability in *Streptomyces lividans* 1326. Appl Microbiol Biotechnol **82:**303-310.

5. **Kieser, T., M. J. Bibb, K. F. Chater, M. J. Butter, and D. A. Hopwood (ed.).** 2000. Practical *Streptomyces* genetics. The John Innes Foundation, United Kingdom, Norwich.

6. **He, Y., Z. Wang, L. Bai, J. Liang, X. Zhou, and Z. Deng.** 2010. Two pHZ1358-derivative vectors for efficient gene knockout in *streptomyces*. J Microbiol Biotechnol **20:**678-682.

7. **Gust, B., G. L. Challis, K. Fowler, T. Kieser, and K. F. Chater.** 2003. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. P Natl Acad Sci USA **100:**1541-1546.

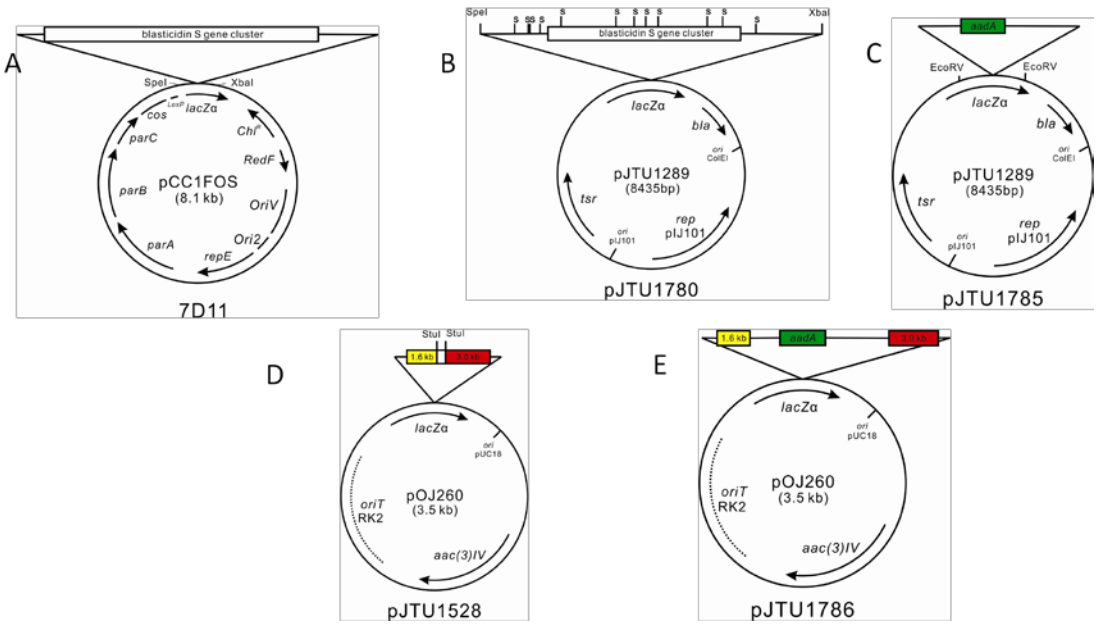

Figure S1 The maps of the plasmids used in the construction of *S. lividans* LL2. A) The plasmid map of 7D11. it contains a 36 kb SpeI-XbaI fragment harboring the intact blasticidin S gene cluster. B) The plasmid map of pJTU1780. It was generated by the insertion of the 36 kb SpeI-XbaI fragment from 7D11 to the SpeI-XbaI site of pJTU1289. The StuI cleavage sites are marked with the letter "S" in the map. C) The plasmid map of pJTU1785. To generate this plasmid, pJTU1780 was first digested by StuI and was then ligated with an blunt-end *aadA* casette. D) The plasmid map of pJTU1528. To generate this plasmid, a 4.85 kb fragment was amplified from *S. lividans* HXY16 and was then inserted into the EcoRV site of pOJ260. The yellow part and the red part of the inserted fragment was used as homologue arms in the generation of *S. lividans* LL1. E) The plasmid map of pJTU1786. To generate this plasmid, a EcoRV fragment from pJTU1785 was ligated with the StuI-digested 1528.

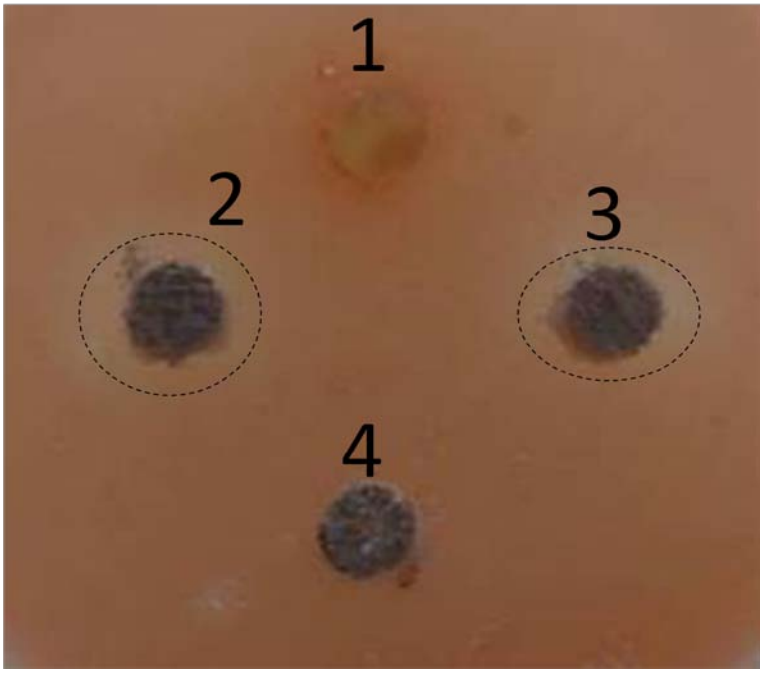

Figure S2 Bioassay of the bls gene cluster engrafted strain, *S. livians* LL2. 1, agar patch; 2 and 3, two independent *S. lividans* LL2 strains; 4, *S. lividans* HXY16.

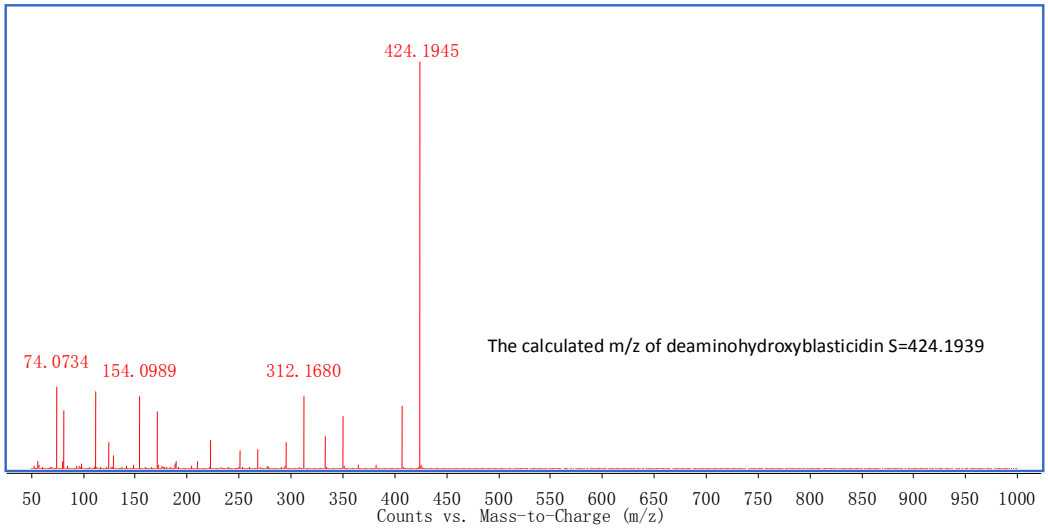

Figure S3 Q-TOF MS confirmation of deaminohydroxyblasticidin S

Figure S4 Protein alignment of SLBSD and its homologue in *S.lividans* TK24.

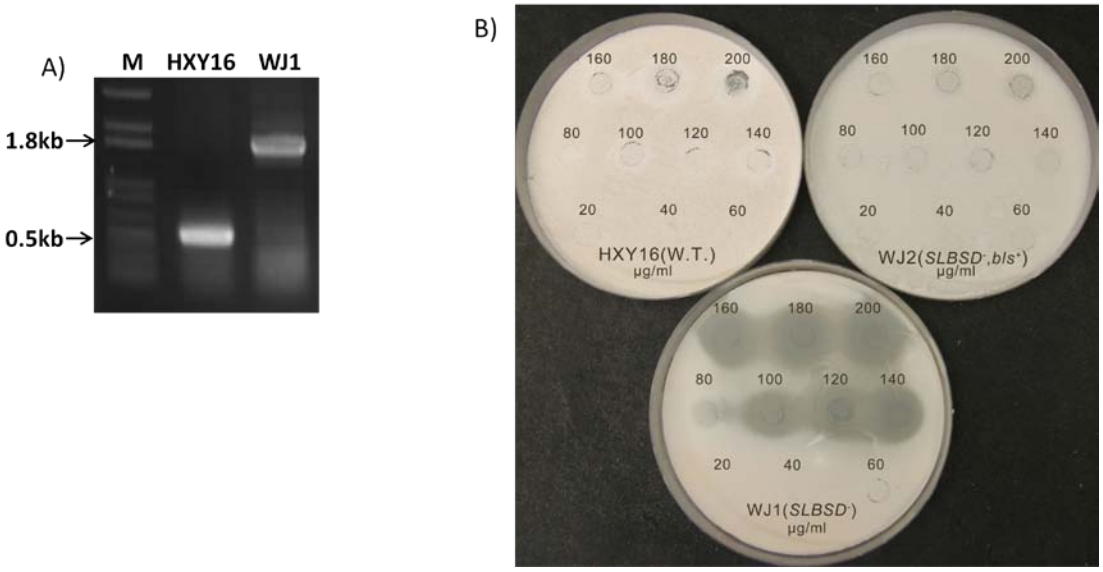

Figure S5 Confirmation of ΔSLBSD mutant *S. lividans* WJ1 and BS resistance experiment of WJ1, WJ2 and HXY16. A) Confirmation of the mutant WJ1 by PCR. M, DNA marker; HXY16, PCR product targeting *SLBSD* from *S. lividans* HXY16; WJ1, PCR product targeting *SLBSD* from *S. lividans* WJ1. B) To test the blasticidin S resistance of each strain, 10 Oxford cups were placed on the SFM medium plates that were pre-inoculated spores of *S. lividans* strains and 25 μl blasticidin S with 10 different concentrations (20 - 200μg/ml) was added in the cups. The concentration of blasticidin S was labeled in the Figure. The inhibition effect of blasticidin S on HXY16 could only weaken the growth of the strain, while the growth of WJ1 was completely inhibited when the blasticidin S concentration was over 100μg/ml.

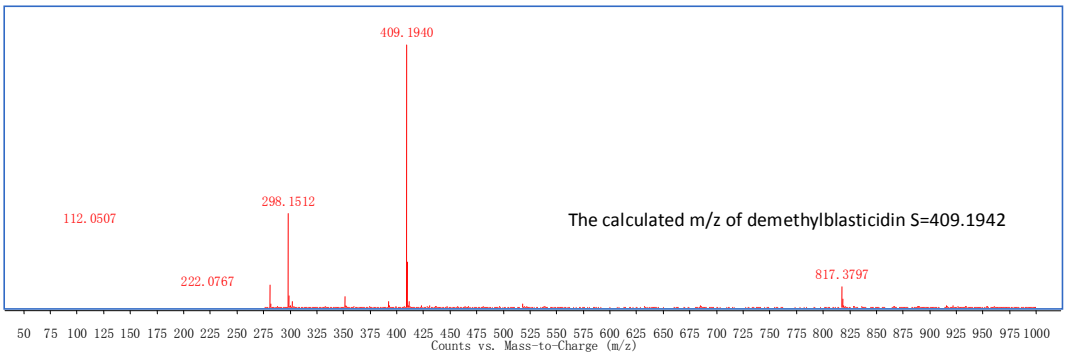

Figure S6 Q-TOF MS confirmation of demethylblasticidin S

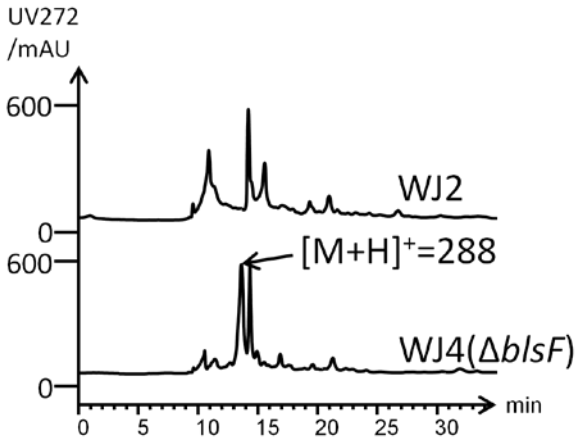

Figure S7 Assay of the blasticidin S production of the ΔblsF mutant strain, S. lividans WJ4.

HPLC comparison of the untreated fermentation broth of WJ4 to that of WJ2.

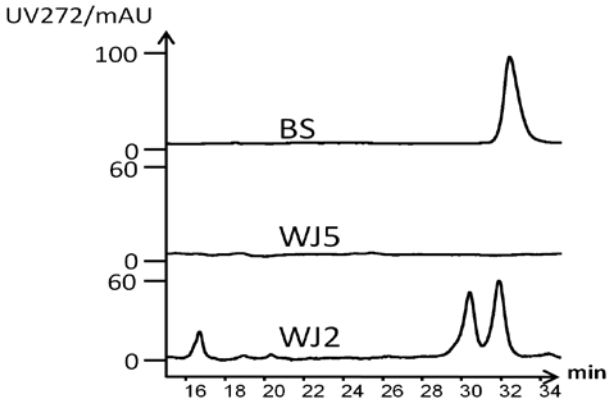

Figure S8 Assay of the blasticidin S production of the Δ*blsE* mutant strain, *S. lividan*s WJ5.

HPLC comparison of the purified fermentation broth of WJ5 to that of WJ2.