## **Supplementary Materials**

## Engineering bafilomycin high-producers by manipulating regulatory and biosynthetic genes in the marine bacterium *Streptomyces lohii*

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**Figure. S1 Multiple protein sequence alignment of BafG with several AfsR family proteins.** The trans\_reg\_C domain, BTA domain and NB-ARC domain are marked with blue, purple and black frames, respectively; three TRP repeats are emphasized by black arrows; the key residues of BafG related to the nucleotides recognition (<sup>24</sup>SerVal<sup>25</sup>, Thr44, Thr47, <sup>55</sup>SerLeu<sup>56</sup>, Thr66 and Gly70) are marked with "•". *Note*: The amino acids highlighted in red stand for 100% homology; the residues highlighted in green and blue stand for 75% < homology <100% and 50% < homology <75%, respectively.

Orf1	MTPSATSEETKPAPRPPRSEPVGDARPAGGDPSAG	35
NarL	DLLKALHQAAAG	149
ORF4	AGTLTASAVTGK. T PDI AGI LVLMDSSGAADAADAGVVT	129
AmphRIV	TVPLTALMVRGG. L PDESSI LVMMPG. AEAESADSEVVS	160
FilF	TVPLTAVAVRGG. L PDTTAI LVMMP TAGDAEGTRVVT	111
FscRI	TLPLNALAVRGCRTPDVAAI LVVMNA. AEEEAGDADVMA	141
GerE	MKEKEF	6
LasR	GALSLSVEAENRAEANRFI ESVLPTLWMLKDYALQSGAGLAFEHP	170
LuxR	GMLSFAHSEKDNYIDSLFLHACMN. PLIVPSLVDNYRKINIANN	177
SalRIII	SGELTGI AVQNTSG NLVGI VVQVRPDEAAAPAAEDAAVK	150
PimM	TVPLTAVAVRGG.LPDTTAILVMMP.SAGDAEGTRVVT	111
ScnRII	TVPLTAVAVRGG.LPDTTAILVMMP.SAGDAEGAHVVT	111
Consensus		
Orf1	TG. VRRLTAVDVRI LEGVAVGTPTVRLAASLYLSRQGVEYRI GLM	79
NarL	ERDVNCLTPRERDILKLIAQGLPNKMARRLDITESTVKVHVKHM	194
ORF4	SQ. KKFLTEI DARI LEGI AAGLSTI PLASRLYLSRQGVEYHVTGL	173
AmphRIV	GRSKKLLSPI DARI LEGI ASGLSTI PLASRLHLSRQGI EYHVTCL	205
FilF	KR. KAI LSAMDARI LEGI AAGVSTVPLASSLYLSRQGVEYHVTCL	155
FscRI	PR. KKLLSEI DARI LEGI AAGVSTI PLASRLYLSRQGVEYHVTGL	185
GerE	QSK. PLUTKREREVFELUVQDKTTKE	50
LasR	VSKPVVLTSREKE <mark>VLQ</mark> WCAI GKTSWE SVI CNCSEANVNFHMGN	215
LuxR	KSN. NOLTKREKECLAWACEGKSSWD SKILGCSERTVTFHLTNA	221
SalRIII	PK. EKLLSSLDAQVLEGVASGASTVQLAARLYLSRQGVEYHVGLM	194
PimM	KR. KKI LSAMDARI LEGI AAGVSTVPLAARLYLSRQGVEYHVTCL	155
ScnRII	KR. KKI LSAMDARI LEGI AAGVSTVPLAARLYLSRQGVEYHVTCL	155
Consensus		
<b>•</b> •	helix-turn-helix motif	
Orf1	MRHFQAANRAALII SIRAHSILGVILSVIGAWPPRVILPEFLE	116
NarL		216
ORF4	L KKL RVP NRAAL VSRAYSMGI L NVG I WPP KVVDDFI K	210
AmphRIV	L KKL RVP NRAAL VSRAYSMGVL KVGVWPP KVVQDFT K	242
	L KKL KVP NRAAL VSRAYSWGVL KVGT VPPEH VDDF VK	192
FSCRI	LKKLKVPNRAALVSRAYSMGVLKVGTWPPKVVEDFTK	222
GerE		/4
LasR		239
		250
SaiRIII	L KKL KAPNKAAL VARAHSWGWL I VGQWPPRVL PEFT K	231
PIMM	L KKL KVPNKAALVSRAYSWGVL KVGI WPPEVVDDFVK	192
SCNRII	EKKEKVIPNKAAEVSRAYSMGVEKVIGI WPPEVVDDFVK	192
Consensus	r	

**Figure. S2 Multiple protein sequence alignment of Orf1 with several LuxR family proteins.** The helix-turn-helix motif is marked with purple frames. *Note*: The amino acids highlighted in red stand for 100% homology; the residues highlighted in green and blue stand for 75% < homology <100% and 50% < homology <75%, respectively.



Figure. S3 The partial open reading frame of BafG (141-210 aa). The "Leu173" shaded in blue is a

unique Leu coded by the rarest codon "TTA" in the Streptomyces genome.



**Figure. S4 Inactivation of** *bafG* and *orf1*. (a) Construction of the *bafG* inactivation mutant *S. lohii*  $\Delta bafG$  (SLO-02). (b) PCR confirmation of the  $\Delta bafG$  mutants. M: DNA marker; WT: *S. lohii* wild type; V: pCIMt002- $\Delta bafG$ ; 1-3: *bafG* inactivation mutants. (c) Construction of the *orf1* inactivation mutant *S. lohii*  $\Delta orf1$  (SLO-03). (d) PCR confirmation of the  $\Delta orf1$  mutants. M: DNA marker; WT: *S. lohii* wild type; V: pCIMt002- $\Delta orf1$ ; 1-3: *orf1* inactivation mutants. *Note*: LA: left homologous arm; RA: right homologous arm.



**Figure. S5 The overexpression of** *bafG/orf1*. (a) Construction of the *bafG* and *orf1* overexpression strains. (b) PCR confirmation of the *S. lohii* mutants. M: DNA marker; WT: *S. lohii* wild type (SLO-01); 1: SLO-06; 2: SLO-04; 3: SLO-05; 4: SLO-07; 5: SLO-08.

 Table S1. The primers for construction of knock-out and regulatory genes overexpression

 vectors.

Primers	Sequence (5'-3')
bafG-LA-FP	AAGAGCTTTTATAAAAGCTT <u>CCATGG</u> GGTGACCGGGCAGGTGAC
bafG-LA-RP	AACGTGAGCCTAGGGCGTGC <u>CCATGG</u> GAGGGAAGCTGCGCAGGA
bafG-RA-FP	TTGGCTGACAATTGACATCT <u>GCTAGC</u> CTGGACGAACTGTGCGCG
bafG-RA-RP	GTGGATCCGCACCCAAGCTTGCCAAGCCCCAAGTCCGATGCG
orf1-LA-FP	AACGTGAGCCTAGGGCGTGCCCATGGTCCCCGGTCCCCCTTCT
orf1-LA-RP	AACGTGAGCCTAGGGCGTGCCCATGGGCCGAACAGGTACCCCAGAC
orf1-RA-FP	TTGGCTGACAATTGACATCT <u>GCTAGC</u> GGAATTCCTTGAACCCTA
orf1-RA-RP	GTGGATCCGCACCCAAGCTTGCTAGCGACGTAGCTGATCAGTTC
BafG-BamHI-FP	TCGTGCCGGTTGGTAGGATCCACGTGCACGTGCACGTGTC
BafG-KpnI-RP	GGGCTGCAGGTCGACTCTAGA <u>GGTACC</u> TCACCCGGCGCGCATGTA
Orf1-BamHI-FP	TCGTGCCGGTTGGTAGGATCCAGGAGGCCGGGGAATGACACCGTC
Orf1-KpnI-RP	GGGCTGCAGGTCGACTCTAGA <u>GG7ACC</u> CTAGGGTTCAAGGAATTC
Spec-NdeI-FP	TCATCTCGTTCTCCGCTCAT <u>CATATG</u> TGTAGGCTGGAGCTGCTTC
Spec-SacI-RP	AATACGAATGGCGAAAAGCC <u>GAGCTC</u> CTGACGCCGTTGGATACAC

*Note:* The italic underline litters indicate the restriction sites.

Primers	Sequence (5'-3')
bafG-KO-FP	GCGGGGTCGATTCCGCCCGG
bafG-KO-RP	ACATCCTCTCGGCCCACGAC
orf1-KO-FP	CCGCGTTCGACCTCAAAGTC
orf1-KO-RP	GTGCATTCACCGCCTTCGGA
M13F-47	CGCCAGGGTTTTCCCAGTCACGAC
M13R-48	AGCGGATAACAATTTCACACAGGA

Table S2. The primers for construction of knock-out vectors and PCR confirmation of *S. lohii* mutants.

Table S3. The primers for quantitative real-time PCR.

Primers	Sequence (5'-3')
hrdB-FP	CCAAGAACCACCTCCTGGAG
hrdB-RP	AGCCCTTGGTGTAGTCGAAC
bafG-FP	CGAGAGGATGTGCCGGTGG
bafG-RP	CTGGTGGGCAAGTTGATGCT
orf1-FP	CCGTCAGCGACCTCTGAAGA
orf1-RP	GACACCTTCCAGGATGCGGA
bafAV-FP	ACTCGCCCGTCGGAGGT
bafAV-RP	GGGACAGGTTGGACTTCAGCG