Supplementary material

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Identification of a Small Molecule Signaling Factor That Regulates the Biosynthesis of the Antifungal Polycyclic Tetramate Macrolactam HSAF in *Lysobacter enzymogenes*

Yong Han,[1,2] Yan Wang,[1,5] Simon Tombosa,[1] Stephen Wright,[1] Justin Huffman,[1] Gary Yuen,[3] Guoliang Qian,[4] Fengquan Liu,[4] Yuemao Shen,[2*] and Liangcheng Du[1*]

(1) Departments of Chemistry, University of Nebraska-Lincoln, NE 68588, USA

(2) Key Laboratory of Chemical Biology, School of Pharmaceutical Sciences, Shandong University, Jinan 250100, China

(3) Department of Plant Pathology, University of Nebraska-Lincoln, NE 68583, USA

(4) College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

(5) College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China

***Liangcheng Du (Corresponding author)**

E-mail: ldu3@unl.edu

***Yuemao Shen (Corresponding author)**

E-mail: yshen@sdu.edu.cn

Table S1. HPLC gradient elution program for HSAF analysis. Mobile phase A: water containing 0.1% formic acid; mobile phase B: acetonitrile containing 0.1% formic acid; flow rate: 1 mL/min; wavelength for detection: 280 nm.

Time (min)	MP A (%)	MP B (%)
0	60	40
4	60	40
17	30	70
20	30	70
25	0	100
28	0	100
29	60	40

Table S2. Sequence of the primers used in this study.

16s-up-realtime PCR	ACTTCGTGCCAGCAGCCG
16s-dw-realtime PCR	CCATTCCCAGGTTGAGCCC
HSAF-*nrps*-up-realtime PCR	GCAGATTCCGCCGCACAT
HSAF-*nrps*-dw-realtime PCR	CGAAGCCGAACGAGTTGACC
clp-up-realtime PCR	CGCCTCTACGACCTGCTGC
clp-down-realtime PCR	CATCGCCTCGGGTTCCTT
rpfG-up-realtime PCR	AGCAGTCCGAGAACGTCAA
rpfG-down-realtime PCR	ATGCGCTCCAGGTAGGC
rpfF-up-realtime PCR	TGGCGCATCTTGTTCATCG
rpfF-down-realtime PCR	TGTGCAAGCGGGTCAGC
rpfC-up-realtime PCR	TGCGCTACGAGACCCATATC
rpfC-down-realtime PCR	GTATCGGTCACGGTGAAACG
rpfB-up-realtime PCR	CGGGGTGTACATCGGATT
rpfB-down-realtime PCR	AGTTCGCCGCCTACCTG
rpfC-up-disruption	GGGGTACCCGAGTGCCTCAACACCATCCA
rpfC-down-disruption	CCCAAGCTTCCGGCCTTCTGCAACAACC
rpfB-up-disruption	TAACTCGAGATTCCAGCCACGAA
rpfB-down-disruption	TATGGATCCATGCTTGAGGATGCGTTC

Table S3. ^{1}H-NMR data of LeDSF3 ($DCCl_3$), δ in ppm, J in Hz.

Position	LeDSF3
2	2.36 (t, J = 7.5 Hz, 2 H)
3	1.63-1.66 (m, 2 H)
4-11	1.27-1.36 (m, 16 H)
12	1.13-1.18 (m, 2 H)
13	1.51-1.54 (m, 1 H)
14	0.88 (d, J = 6.6 Hz, 6 H)

Figure S1. ^{1}H-NMR of LeDSF3

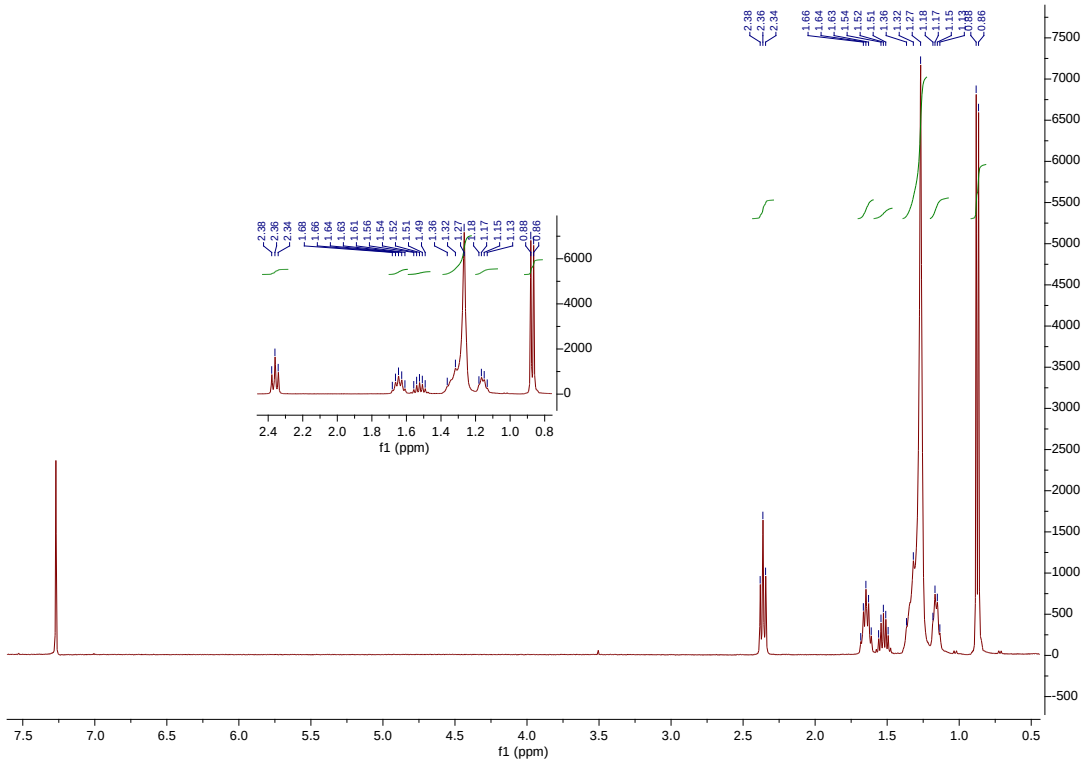


Figure S2. ^{13}C-NMR of LeDSF3

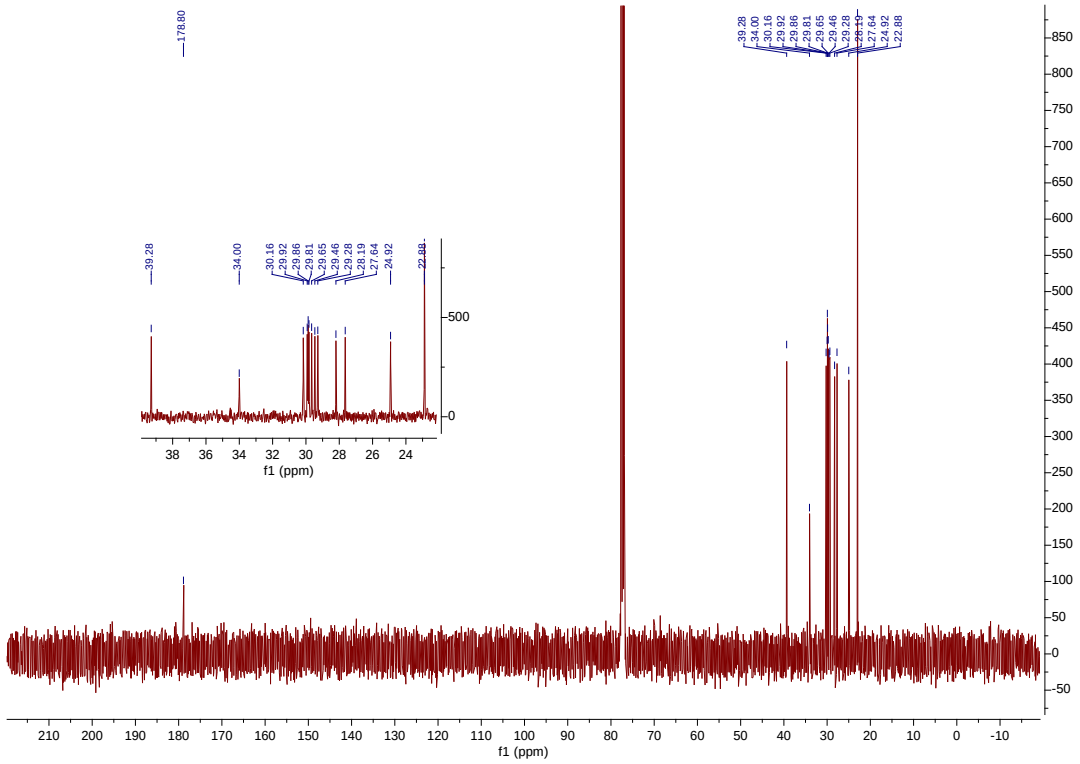


Figure S3-1. MS of LeDSF3 (negative mode)

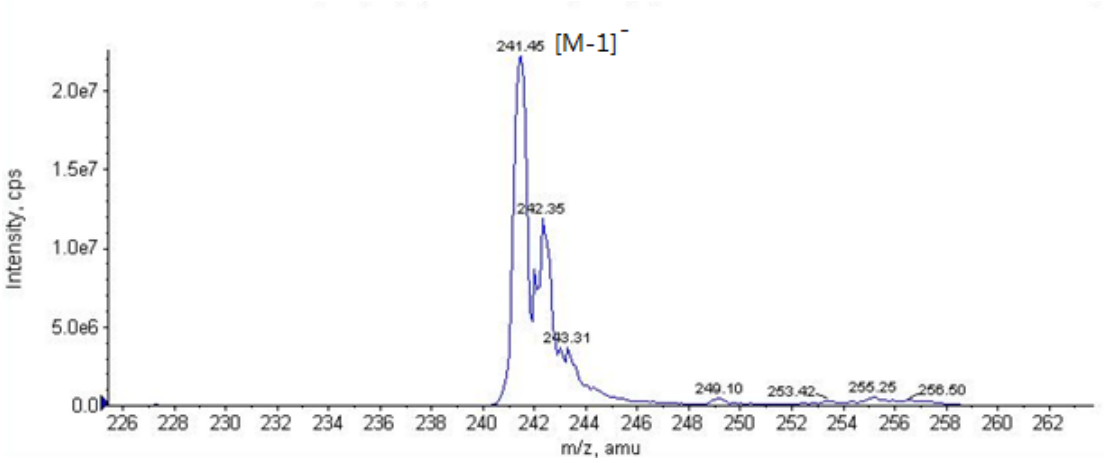


Figure S3-2. MS of LeDSF3 (positive mode)

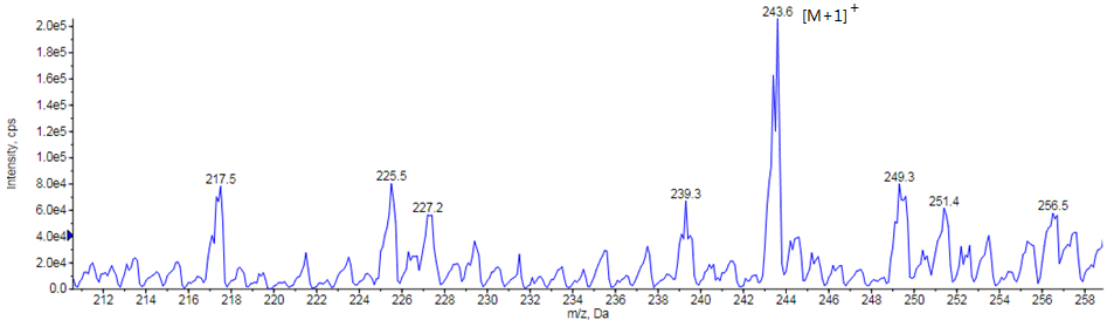


Figure S3-3. MS-MS of LeDSF3 (MS2)

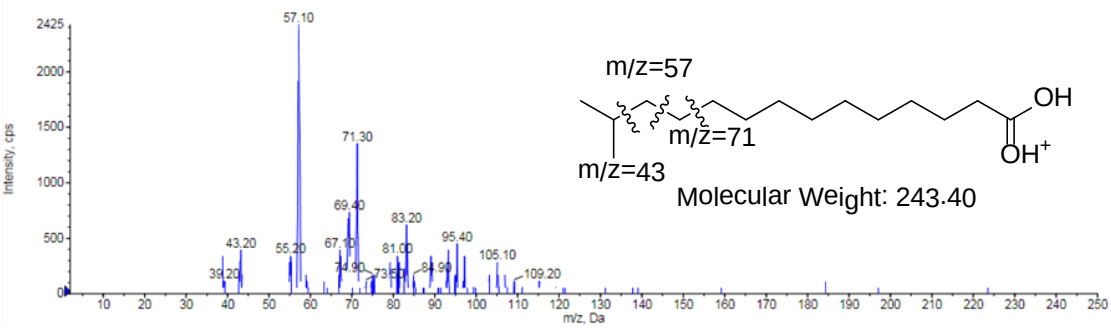


Figure S4. IR of LeDSF3

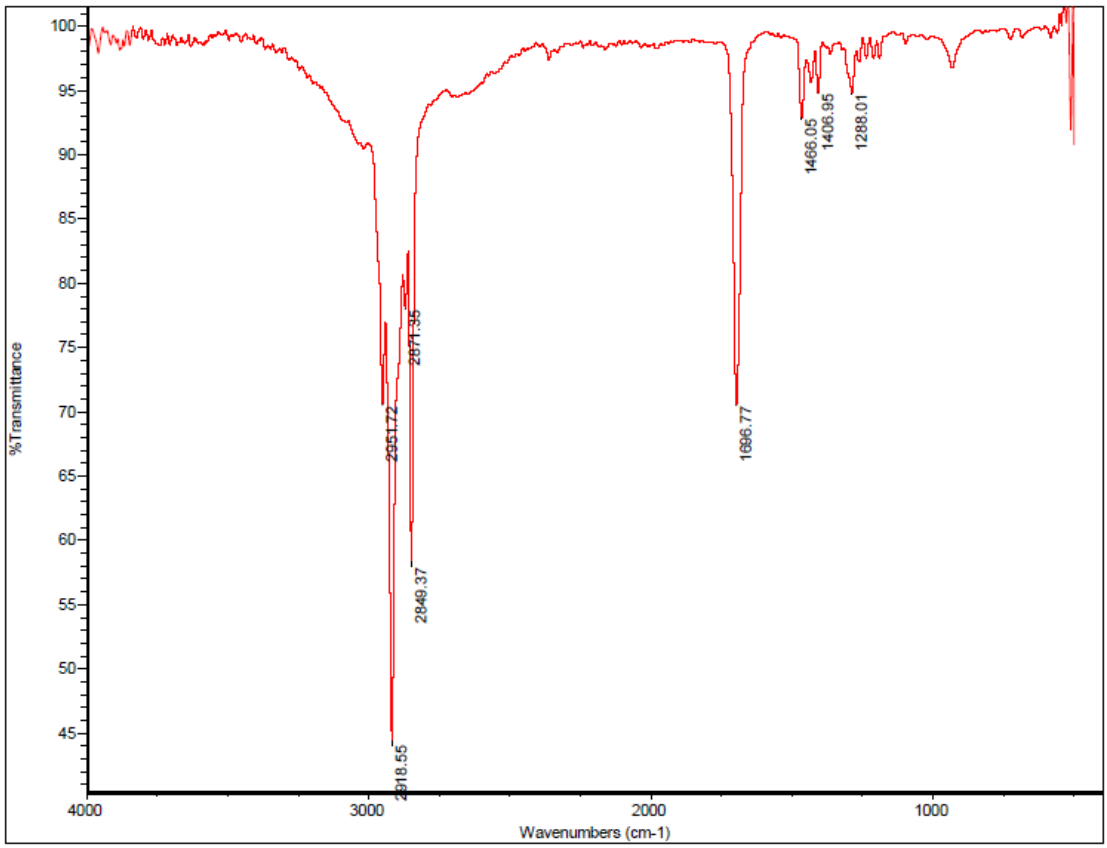
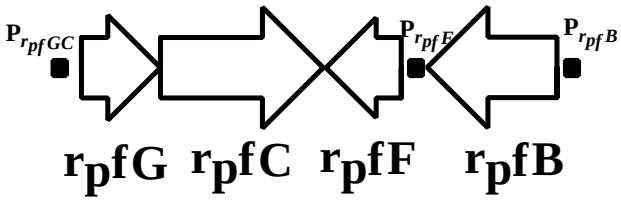


Figure S5. The *rpf* gene cluster and predicted promoters in *L. enzymogenes* OH11.



P_{rpfB}:CGGGCACCGCCTTGCCACCCGCTAGAGCCTATGCTCCAAT**C**GAGAAAACA

P_{rpfF}:GAGGGGCTTTTATTTTTGCGGCGGAATCGGGGTGTAGCAT**C**GATGCGTGG

P_{rpfGC}:GCTGGTCGATGACCGACGCGGTGCTGGAGCACAATCCGGA**G**ATCAAGCGC

Figure S6. HSAF production in various strains of *L. enzymogenes*. **A:** the wild type *Le*OH11; **B:** *Le*OH11*ΔrpfB*; **C:** *Le*OH11*ΔrpfC*; **D:** *Le*OH11*ΔrpfF*; **E:** *Le*OH11*ΔrpfG*.

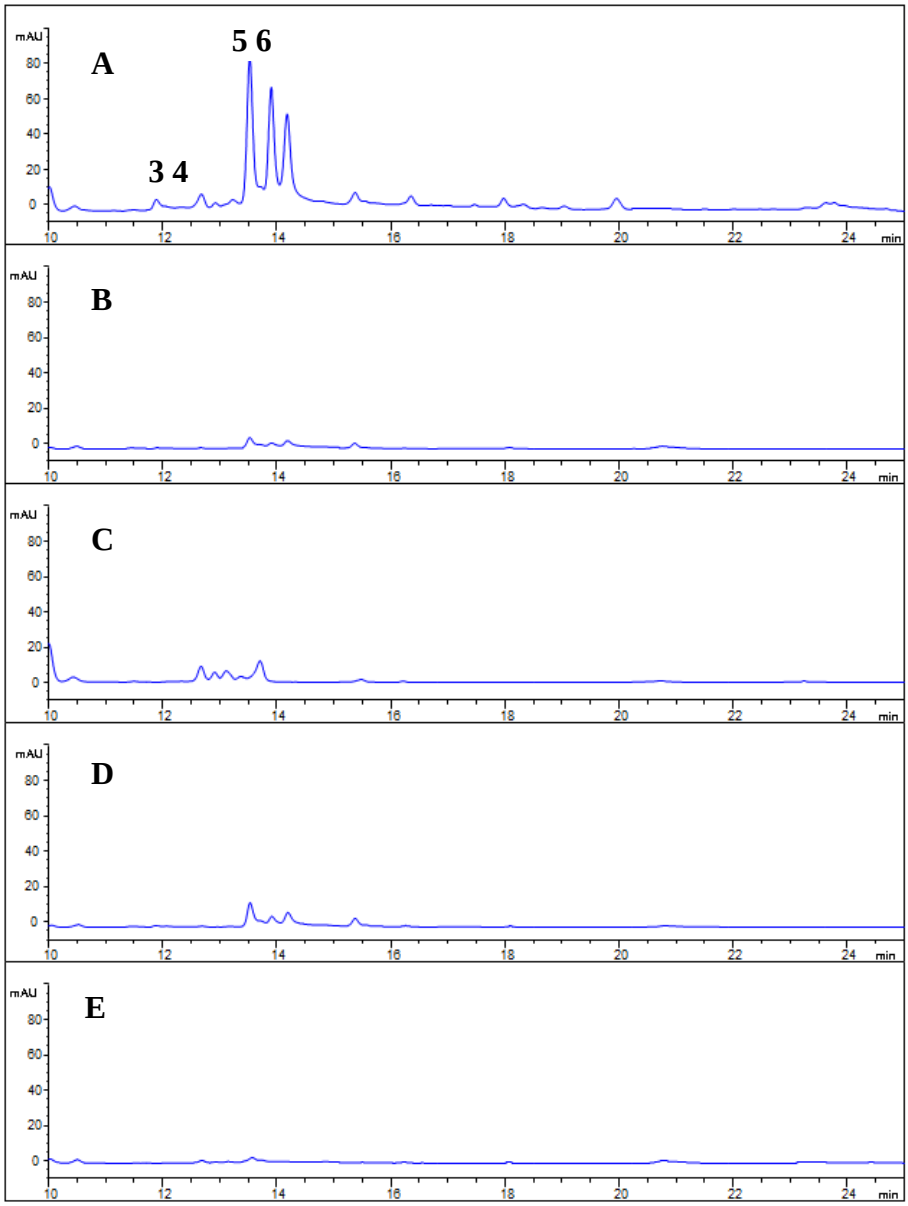


Figure S7. Antifungal activity of *Le*OH11 and various mutants against *Fusarium verticillioides*. On each of the petri dishes (**A** through **J**), *Le*OH11 was spread over the left half of the dish, and *F. verticillioides* was point-inoculated in the center of the right half of the same dish. The blue line was drawn to show the two halves of the same dish. **A** and **F:** the wild type *Le*OH11; **B** and **G:** *Le*OH11*ΔrpfB*; **C** and **H:** *Le*OH11*ΔrpfC*; **D** and **I:** *Le*OH11*ΔrpfF*; **E** and **J:** *Le*OH11*ΔrpfG*. For all dishes, **A** through **E** show the antifungal activity in the absence of *Le*DSF3, and **F** through **J** the activity in the presence of 5 μM *Le*DSF3.