Activation and molecular mechanism of a cryptic oviedomycin biosynthetic gene cluster via the disruption of a global regulatory gene--*adpA* in *Streptomyces ansochromogenes*

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Supplementary Table 1: Examples of OvmZW-like proteins from different strains

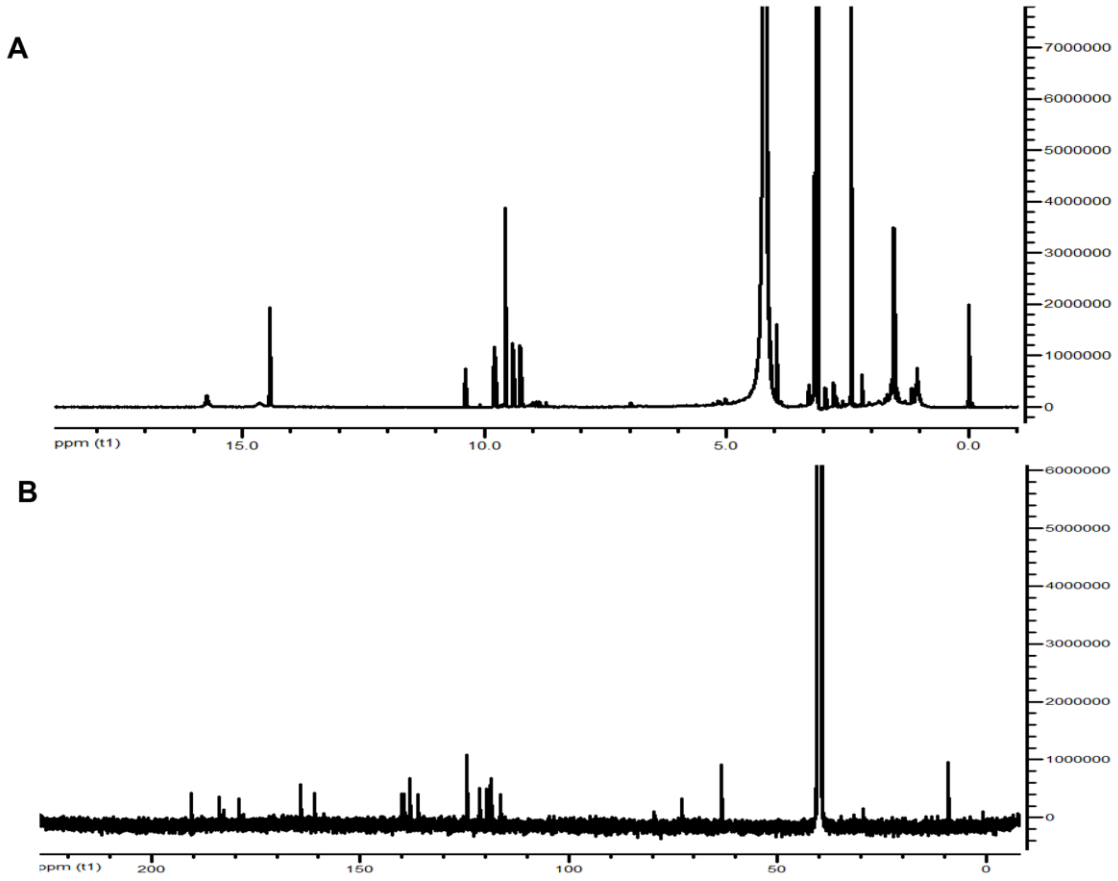
Strains	Identity with OvmZ	Identity with OvmW	Cluster type
Streptomyces alni	40%	79%	T2pks
Streptomyces fradiae	40%	62%	T1pks
Streptomyces fulvissimus	47%	78%	T2pks
Streptomyces sp. Ag109_O5-10	49%	81%	T2pks
Streptomyces sp. CCM_MD2014	45%	75%	T2pks
Streptomyces sp. DvalAA-19	49%	77%	T2pks
Amycolatopsis halophila	42%	72%	Other
Kitasatospora sp. HKI 714	34%	65%	Phenazine
Nocardia crassostreae	40%	74%	Terpene
Nocardia vulneris	35%	71%	Lantipeptide-Terpene

Supplementary Table 2: Primers used in this study

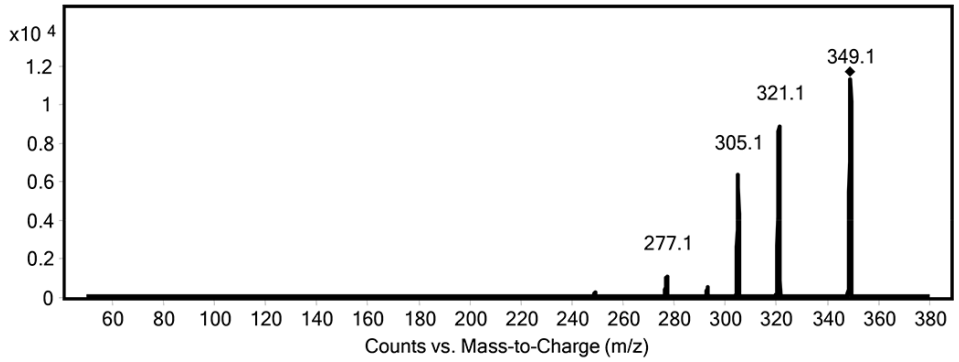
Primers	Sequence (5'-3')	Purpose
UZF	AAAAAGCTTAACACCGACCTGAGGGCACA	*ovmZ* disruption
UZR	AAACATATGGTTTGTCGGACCCGTCGTT	*ovmZ* disruption
DZF	AAACATATGAGGAGGCCGCCCGATGA	*ovmZ* disruption
DZR	AAATCTAGATCGGTGGTGGACGAACA	*ovmZ* disruption
UWF	AAAGAATTCACGCTGCTGCTCACGGTGGT	*ovmW* disruption
UWR	AAAAAGCTTTGACGACCGCTCACGGTCAG	*ovmW* disruption
DWF	AAAAAGCTTGAGTTCCGTGGGCACGAAGC	*ovmW* disruption
DWR	AAATCTAGACGAAACCGCGTGCATGTCCAT	*ovmW* disruption
URF	AAAAAGCTTGATGATGTTCGGCATCGCGA	*ovmR* disruption
URR	AAATCTAGACGAAACCGCGTGCATGTCCAT	*ovmR* disruption
DRF	AAATCTAGAATCGGCCTGGAGGAGTGCATCG	*ovmR* disruption
DRR	AAAGAATTCGACGTCGTAGCGGATGTCCA	*ovmR* disruption
UYF	AAAGAATTCACGCTGCTGCTCACGGTGGT	*ovmY* disruption
UYR	AAAAAGCTTGCTTCCGGTCACCTGAAGTCA	*ovmY* disruption
DYF	AAAAAGCTTGATGATGTTCGGCATCGCGA	*ovmY* disruption
DYR	AAATCTAGACGAAACCGCGTGCATGTCCAT	*ovmY* disruption
UOrf3F	AAAGAATTC CTCGCCAACGTCGTGGACGAC	*orf3* disruption
UOrf3R	AAAAAGCTTGCTAGATGGGACGGTTCGCAC	*orf3* disruption
DOrf3F	AAAAAGCTTGATCTCCACCGTGGCGAGGT	*orf3* disruption
DOrf3R	AAATCTAGACACCGGTCACCAGGATCATCC	*orf3* disruption
UOrf4F	AAATCTAGA AGGCGATCCGTGACCTGGGT	*orf4* disruption
UOrf4R	AAAGAATTCGACCTGGAGGGAGCAGTCCACA	*orf4* disruption
DOrf4F	AAAGAATTCGACCTGCTTCATGATCACGT	*orf4* disruption
DOrf4R	AAAAAGCTTAGCATCATCGTAAGAACGT	*orf4* disruption
ad1-F	TCGTCGCGGTGCTGCTGTT	*adpA* disruption
ad1-R	CCACCGGGGAGCGGAAGC	*adpA* disruption
ad2-F	GGAATTCTGTCTCGGCGTTGTGAT	*adpA* disruption
ad2-R	GGGGTACCGGCTCCTCGGTTGTGT	*adpA* disruption
hrdBpF	GTCGACTCTAGACCGCCTTCCGCCG	*hrdB* promoter for over-expression
hrdBpR	GAACAACCTCTCGGAACGTTGA	*hrdB* promoter for over-expression
ovmZF	ATGGCCGGCCAGCCACGACCCAA	*ovmZ* over-expression
ovmZR	AAAGAATTCTCATCGGGCGGCCTCCTC	*ovmZ* over-expression
ovmWF	ATGAGCCGTACGCCACGGCGGCGCTT	*ovmW* over-expression
ovmWR	AAAGAATTC TCAGCCGAGGGCCTCGGA	*ovmW* over-expression
ovmRF	ATGGACATGCACGCGGTTTC	*ovmR* over-expression
ovmRR	AAAGAATTCTCGTTCACATGACGCGGTGGT	*ovmR* over-expression
hrdBpF'	GACTCTAGAGGATCCGCGGCCGCGCGCGATC GAGCACTGACCGCCTTCCGC	*hrdB* promoter for heterologous expression
hrdBpR'	GGTGTCTCCTTGGAACGTTGAAAAACGGCTT CCGGC	*hrdB* promoter for heterologous expression
F1F	GCCGGAAGCCGTTTTTCAACGTTCCAAGGAG ACACCATGGACGCTGCCCTCGTCGAC	*ovm* heterologous expression
F1R	GGTGCCCCTGCTCGGCCAGCAGCCGGGCCA	*ovm* heterologous expression
F2F	TGGCCCGGCTGCTGGCCGAGCAGGGGCACC	*ovm* heterologous expression

F2R	CCCGGGACCGCGGGACGGCCGTGCGGACCG	*ovm* heterologous expression
F3F	CGGTCCGCACGGCCGTCCCGCGGTCCCGGG	*ovm* heterologous expression
F3R	AAACAGCTATGACATGATTACGAATTCGATT CCCGTCAGCCGGTGATCTCG	*ovm* heterologous expression
ovmOIPF	TCCTGCCGGACTCCGCGCACGT	Probe *ovmOI* for EMSA
ovmOIPR	CCGCGCCTCCTTTCCTGTTGC	Probe *ovmOI* for EMSA
OIF	GGTCATCGTGGGAGCAGG	Probe for EMSA as a negative control
OIR	CCTTGACGCTGAAGTGGG	Probe for EMSA as a negative control
P_{ovmOI}F	AAACATATGTCCTGCCGGACTCCGCGCACGT	Promoter *ovmOI* for *gusA* reporter plasmid construct
P_{ovmOI}R	CCGCGCCTCCTTTCCTGTTGC	Promoter *ovmOI* for *gusA* reporter plasmid construct
gusAF	ATGACCGGTCTGCGGCCCGTCGAA	*gusA* reporter plasmid construct
gusAR	AAAGCGGCCGCCTCACTGCTTCCCGCCCTGC T	*gusA* reporter plasmid construct
tfdF	AAACATATGGACTCACCCGTCGTCGCGCGCT	*gusA* reporter plasmid construct
gusA2R	AAAACTAGTCTCACTGCTTCCCGCCCTGCT	*gusA* reporter plasmid construct
ovmZPF	TTTGTCGGACCCGTCGTT	Probe *ovmZ* for EMSA
ovmZPR	TCCTTCGGCCGAAACCGCGT	Probe *ovmZ* for EMSA
T7ter	AAAGAATTCGCTAGTTATTGCTCAGCGGTG	*ovmZ* and *ovmW* over-expression with His_6 tag
M13F	GGCCGCTCTAGAACTAGTGGATCC	Probe P_{ovmZ} for DNaseI footprinting
M13R	GGTCGACGGTATCGATAAGCTTG	Probe P_{ovmZ} for DNaseI footprinting
pks1-F	CACCGCCTCCGTCTACGAAGCACA	*pks1* RT-PCR
pks1-R	GGCGCATCCGGCATCCCTCCAT	*pks1* RT-PCR
pks2-F	CGGGCAGATGTACCACGAC	*pks2* RT-PCR
pks2-R	CACCAGCGAGGAGGAGCAG	*pks2* RT-PCR
pks3-F	CTGCGAGTTCGGCGGCTAC	*pks3* RT-PCR
pks3-R	TCGGCGGTACGACCTGCTT	*pks3* RT-PCR
pks4-F	GGTGCTGGCCCTGATACGC	*pks4* RT-PCR
pks4-R	GTGCCCGAGGTTGGACTTGA	*pks4* RT-PCR
pks5-F	AAGGACCCGTCGTCACCG	*pks5* RT-PCR
pks5-R	CCGTTCCAGCAGCAGCAT	*pks5* RT-PCR
pks6-F	TGTGCAAGCCCTCGGTGTC	*pks6* RT-PCR
pks6-R	CACGTCGATGTCGCTGGTG	*pks6* RT-PCR
pks7-F	AGGGCTCCGCCTTCTTCGT	*pks7* RT-PCR
pks7-R	CCGGTCGTTCTGCTTGGTG	*pks7* RT-PCR
pks8-F	CGGTGGTCAACTCCCTCTTC	*pks8* RT-PCR
pks8-R	TCGGTCGTCCCAGTCGTAG	*pks8* RT-PCR
nrps1-F	CATCGACAGCCAGGTGAAGC	*nrps1* RT-PCR
nrps1-R	GTAGGCGGGCAGGTAGGAG	*nrps1* RT-PCR
nrps2-F	CGGTGAGTGCATCGACATCC	*nrps2* RT-PCR
nrps2-R	CCGTAGGCGTCCACGAGAAT	*nrps2* RT-PCR
nrps3-F	GGACACCTACGGGCTCACC	*nrps3* RT-PCR
nrps3-R	CACAGCACCAGACGCAAGG	*nrps3* RT-PCR

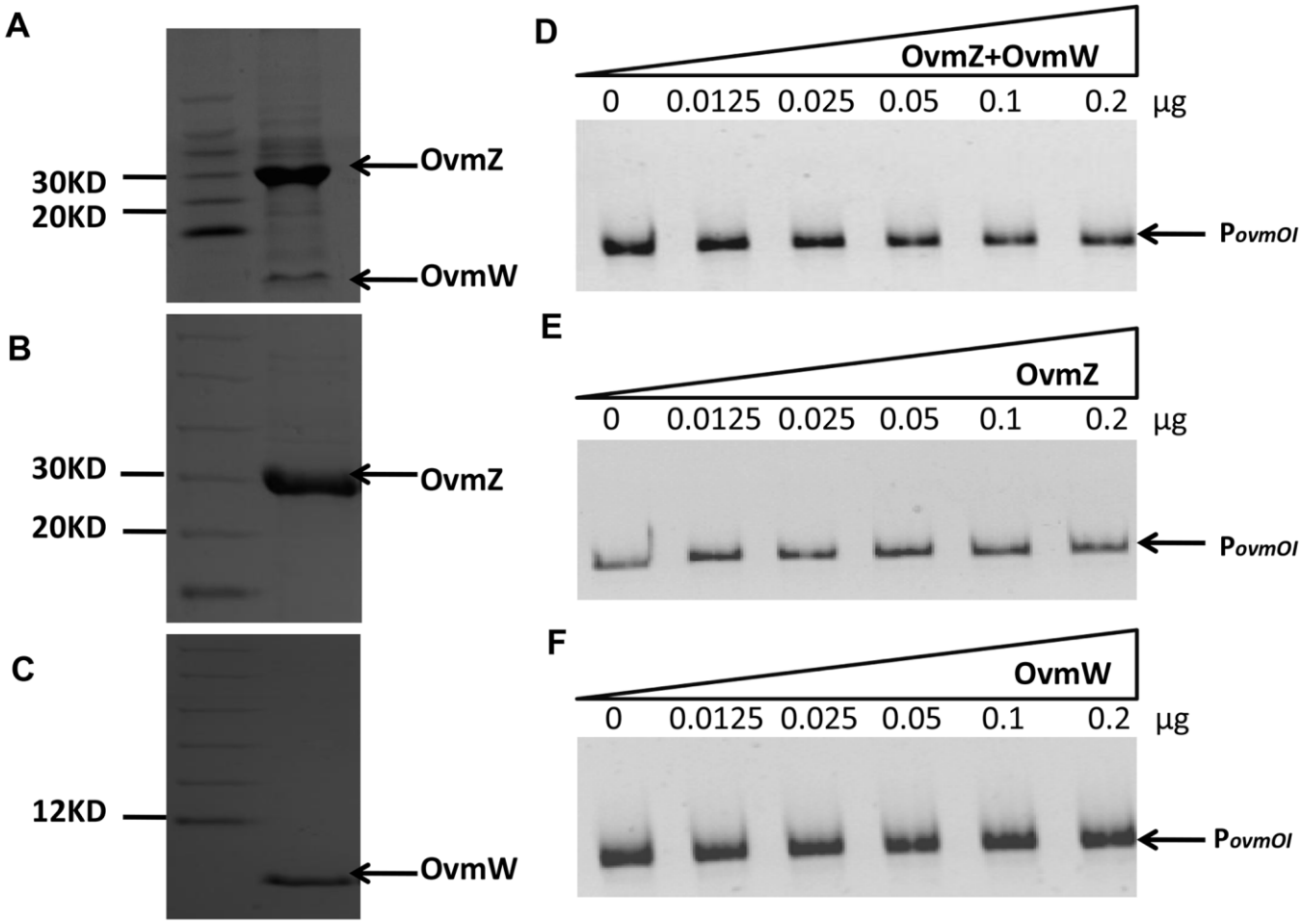
nrps4-F	CTACCACGCCGAGACCACC	*nrps4* RT-PCR
nrps4-R	CTGCTGAAGTCCCGCCAAG	*nrps4* RT-PCR
nrps5-F	CGGTGGTGAACGCCGAGTA	*nrps5* RT-PCR
nrps5-R	CCGAACAGGGTCAGCAGGA	*nrps5* RT-PCR
pks-nrps1-F	GGGGCCTGATCGGCTTCTT	*pks-nrps1* RT-PCR
pks-nrps1-R	GCACCTCGGCGTGTTCCAT	*pks-nrps1* RT-PCR
pks-nrps2-F	CGGGAGAAGGTCGGCTACA	*pks-nrps2* RT-PCR
pks-nrps2-R	GGGTTGGGACGGGAGAAGT	*pks-nrps2* RT-PCR
sanO-F	CGGCGCTGGAGGAACGTAC	*sanO* RT-PCR
sanO-R	GGGTGTAGAGGCCGATGCT	*sanO* RT-PCR
qRT-ZF	CGCGGAGCTTTACGAAGAAT	*ovmZ* Q-PCR
qRT-ZR	CTGCGTTTCCGTCCGTATC	*ovmZ* Q-PCR
qRT-WF	TTCGTGCCCACGGAACT	*ovmW* Q-PCR
qRT-WR	GATGAGTTCGTCCAGGTCGT	*ovmW* Q-PCR
qRT-RF	CAGCAGGCGTTCCTCAA	*ovmR* Q-PCR
qRT-RR	GAAGACGCCCTCGTAGATG	*ovmR* Q-PCR
qRT-orf3F	ATGCGAACGACGGAGGAG	*orf3* Q-PCR
qRT-orf3R	ACGATCTGCAGCCGTACC	*orf3* Q-PCR
qRT-orf4F	AACTCTGCCAGCGGATTC	*orf4* Q-PCR
qRT-orf4R	AGCAGCTCGATCTCCCA	*orf4* Q-PCR
qRT-OIF	GCATCTCGCTGCCGCTGA	*ovmOI* Q-PCR
qRT-OIR	CGTCAACCTGGGCTGGAAGC	*ovmOI* Q-PCR
qRT-PF	AGGGCTCCGCCTTCTTCGT	*ovmP* Q-PCR
qRT-PR	TCATGTGGTACGCGTTGGA	*ovmP* Q-PCR
qRT-SF	GGCCACTCAGGAATTCACC	*ovmS* Q-PCR
qRT-SR	AACTCGGAGTCGAGGAAGT	*ovmS* Q-PCR
qRT-TF	GGACGTGCTGGTGAACAA	*ovmT* Q-PCR
qRT-TR	CTGTTGAGGTTGGTGTCGAT	*ovmT* Q-PCR



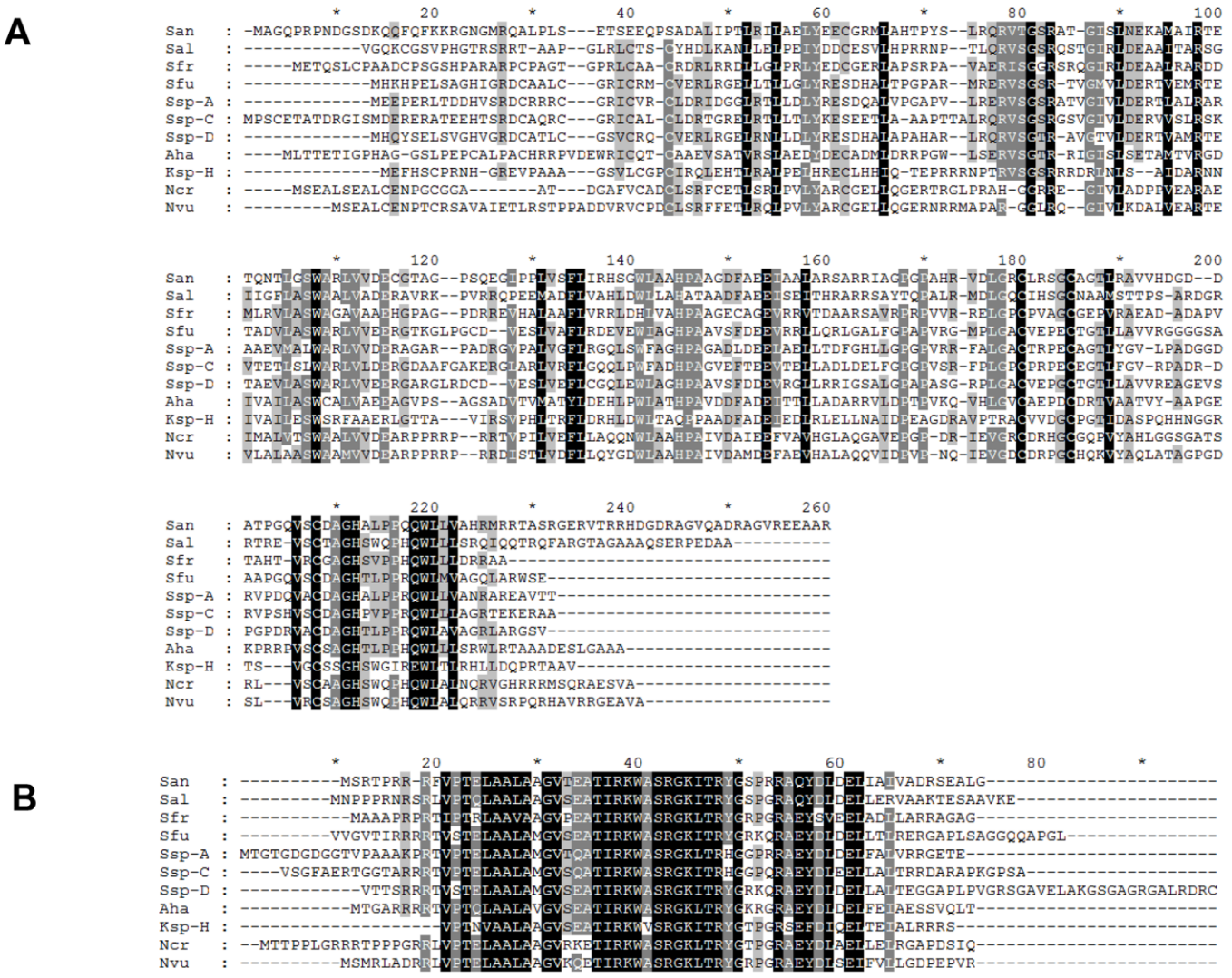
Supplementary Figure 1. NMR analysis of the new bioactive compound. *A*, ^{1}H NMR. *B*, ^{13}C NMR.



Supplementary Figure 2. MS/MS analysis of oviedomycin generated by heterologous expression in *S. coelicolor* M1146.



Supplementary Figure 3. EMSAs to determine the potential targets of OvmZW. *A-C*, SDS-PAGE of purified OvmZ, OvmW and OvmZW. Samples were separated by 12% SDS-PAGE and stained with Coomassie brilliant blue R-250. *D-F*, EMSAs of OvmZ, OvmW and OvmZW with the probe P_{ovmOI} containing the promoter of *ovmOI*. Each lane contains 20 ng DNA probes.



Supplementary Figure 4. Alignment of OvmZ and OvmW-like regulators from different strains. *A*, Alignment of OvmZ-like regulators from different strains. *B*, Alignment of OvmW-like regulators from different strains. San: *Streptomyces ansochromogenes*, Sal: *Streptomyces alni*, Sfr: *Streptomyces fradiae*, Sfu: *Streptomyces fulvissimus*, Ssp-A: *Streptomyces* sp. Ag109_O5-10, Ssp-C: *Streptomyces* sp. CCM_MD2014, Ssp-D: *Streptomyces* sp. DvalAA-19, Aha: *Amycolatopsis halophila*, Ksp-H: *Kitasatospora* sp. HKI 714, Ncr: *Nocardia crassostreae,* Nvu: *Nocardia vulneris*.

Supplementary Method

***Bioassays for anticancer activity*:** Human lung carcinoma cell line A549, human hepatocellular carcinoma cell line HepG2 and human breast adenocarcinoma cell line MCF-7 were purchased from the Cell Bank of Chinese Academy of Sciences, Shanghai, China, and were cultured and propagated according to the procedures provided by the supplier. Confluent cells grown in 75 cm^2 flasks were collected and seeded in a 96-well plate and cultured for 24 h at 37 ˚C in a humid incubator containing 5% CO_2. Then the cells were exposed to different concentrations of oviedomycin for 72 h. 20 microliters of 5 mg/ml thiazolyl blue tetrazolium bromide (MTT) in phosphate buffered saline was added into each well followed by 4 h incubation. The supernatant was removed and 150 μl dimethyl sulfate was added to dissolve the reduced formazan, and the absorption at 570 nm was recorded on a microplate reader. The assays were performed in four replicates for IC_{50} calculation based on the fraction of viable cells.