The critical role of polyketide synthase gene on the swainsonine biosynthesis in the fungus *Metarhizium anisopliae*

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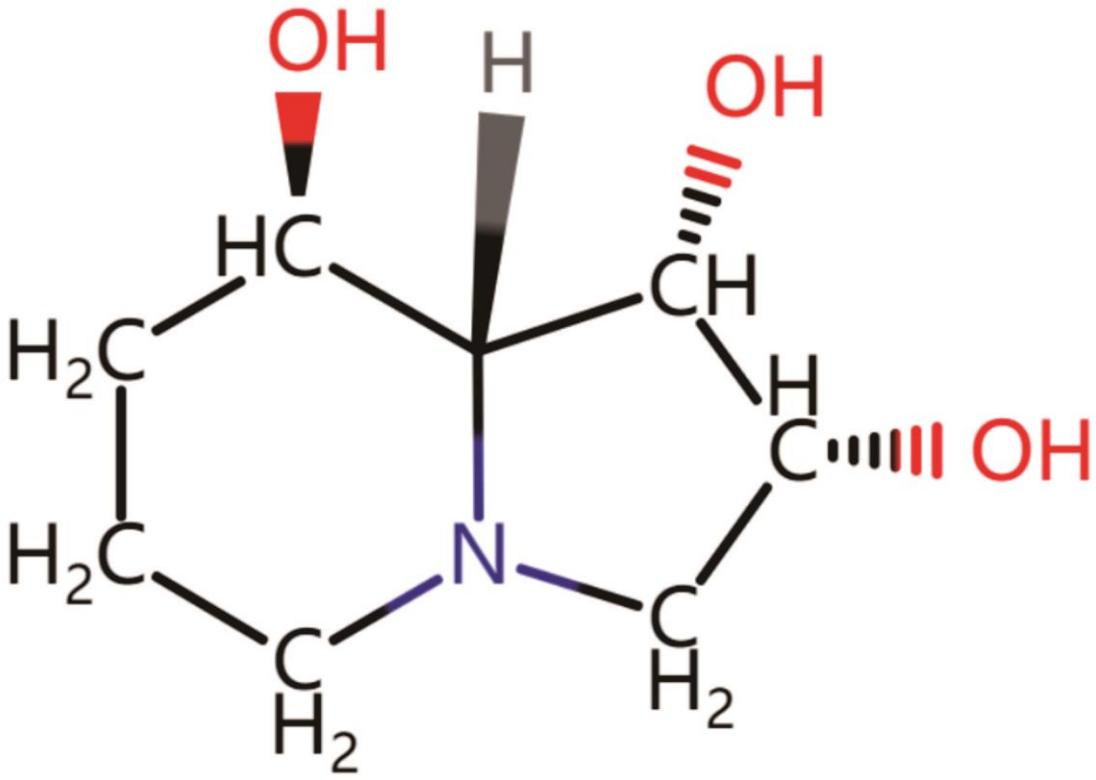
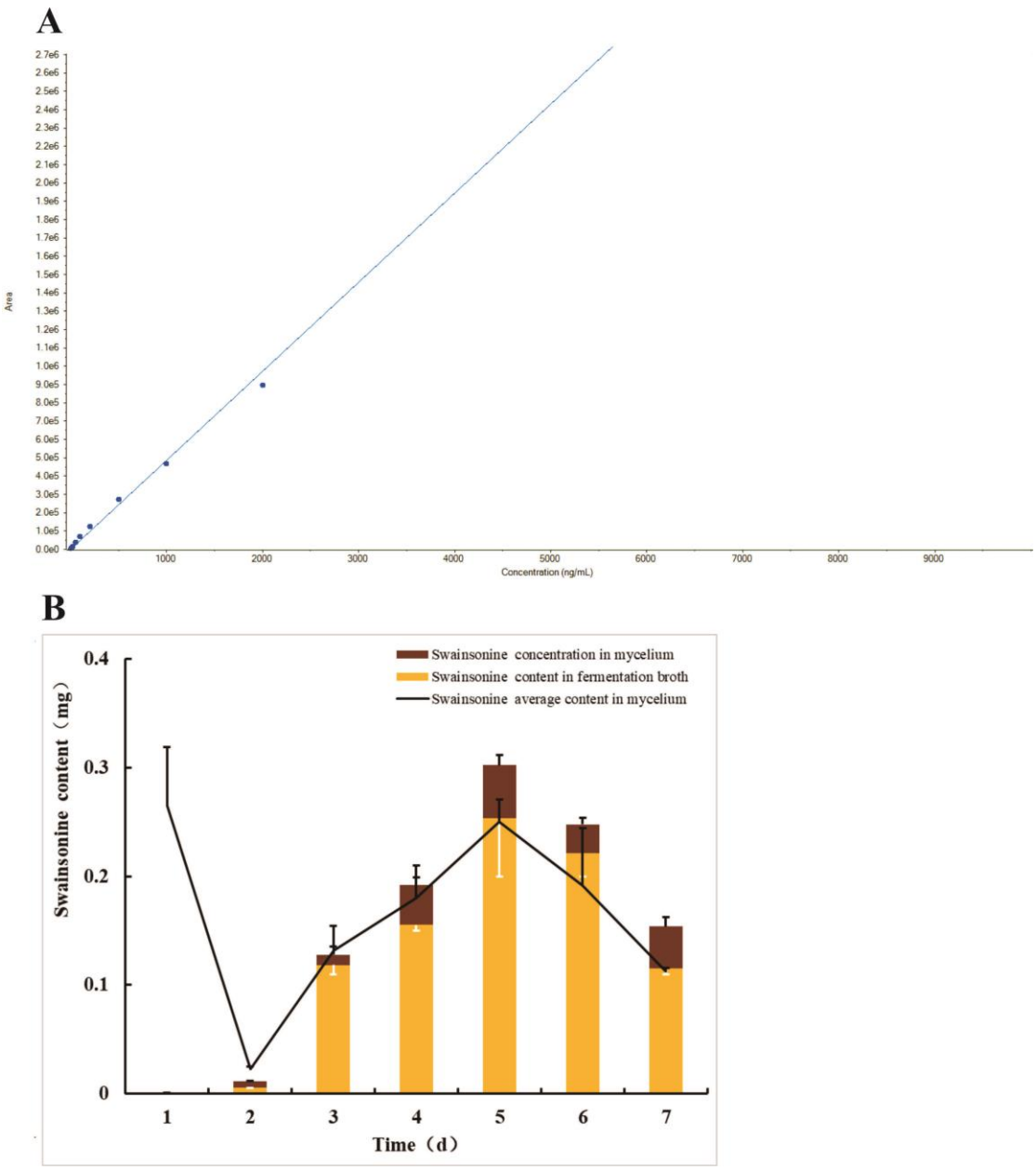
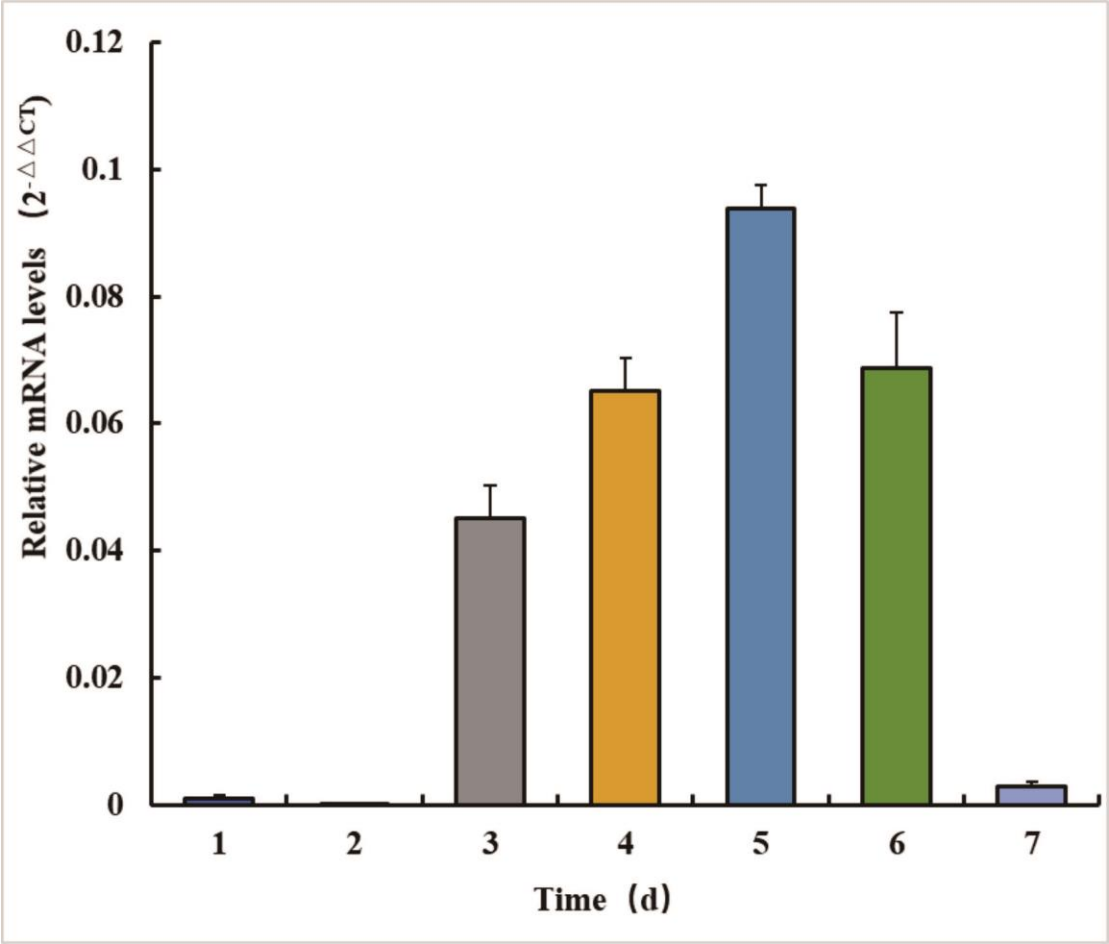


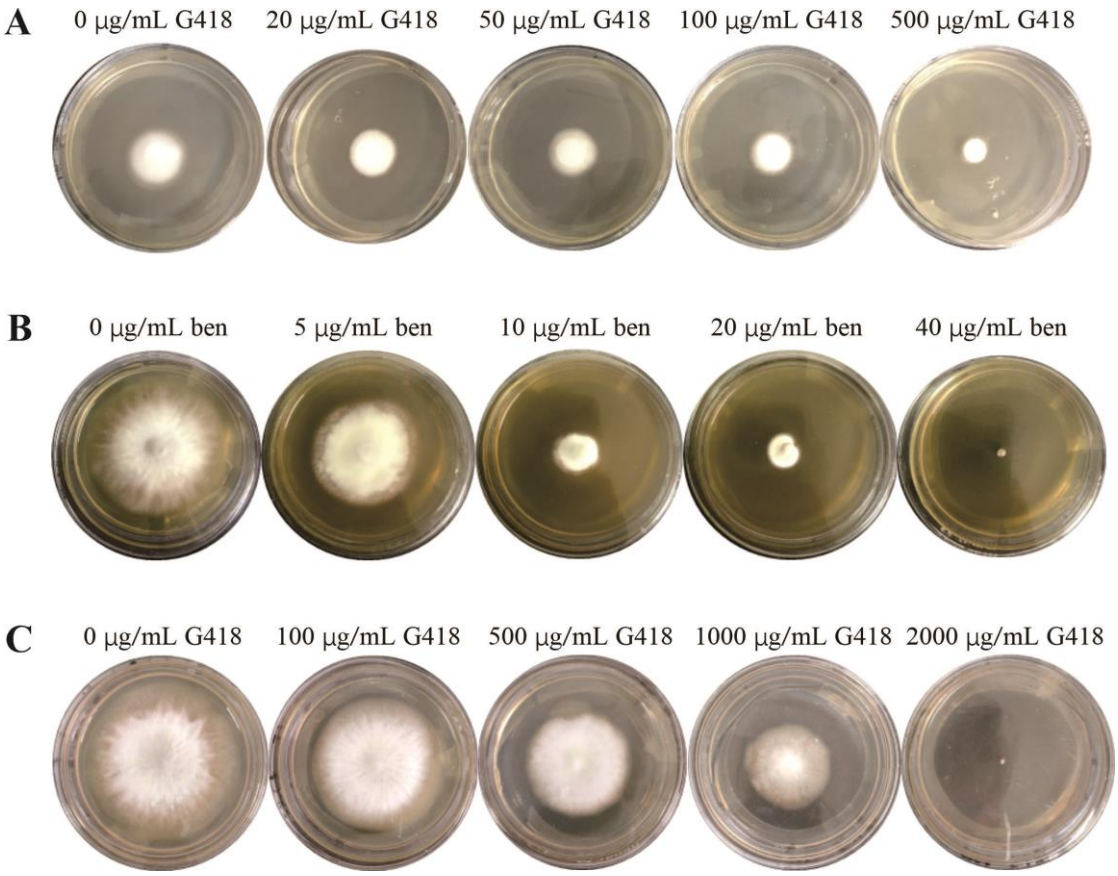
Supplementary Figure 1. Swainsonine chemical structure.



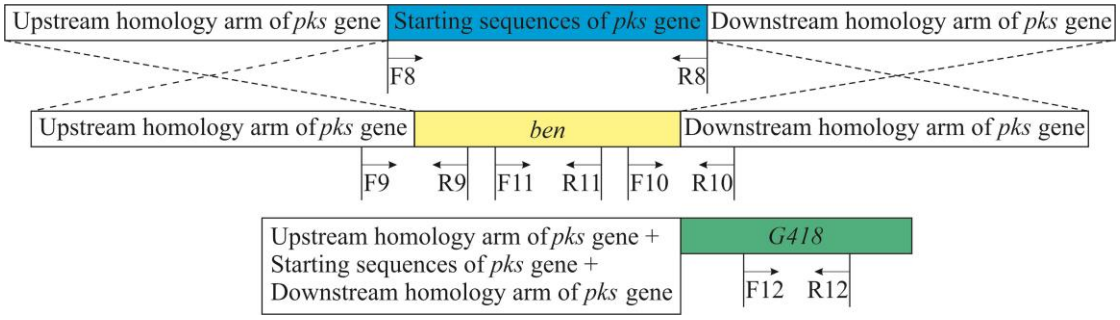
Supplementary Figure 2. Detection of SW content of *M. anisopliae* in different time. A: The standard curve was drawn according to the calculated regression equation: Y=486.14665X+34.36851 (r=0.99049). B: The content of swainsonine of *M. anisopliae* from 1st to 7th d. Error bars represent the standard error of the mean (n = 3).



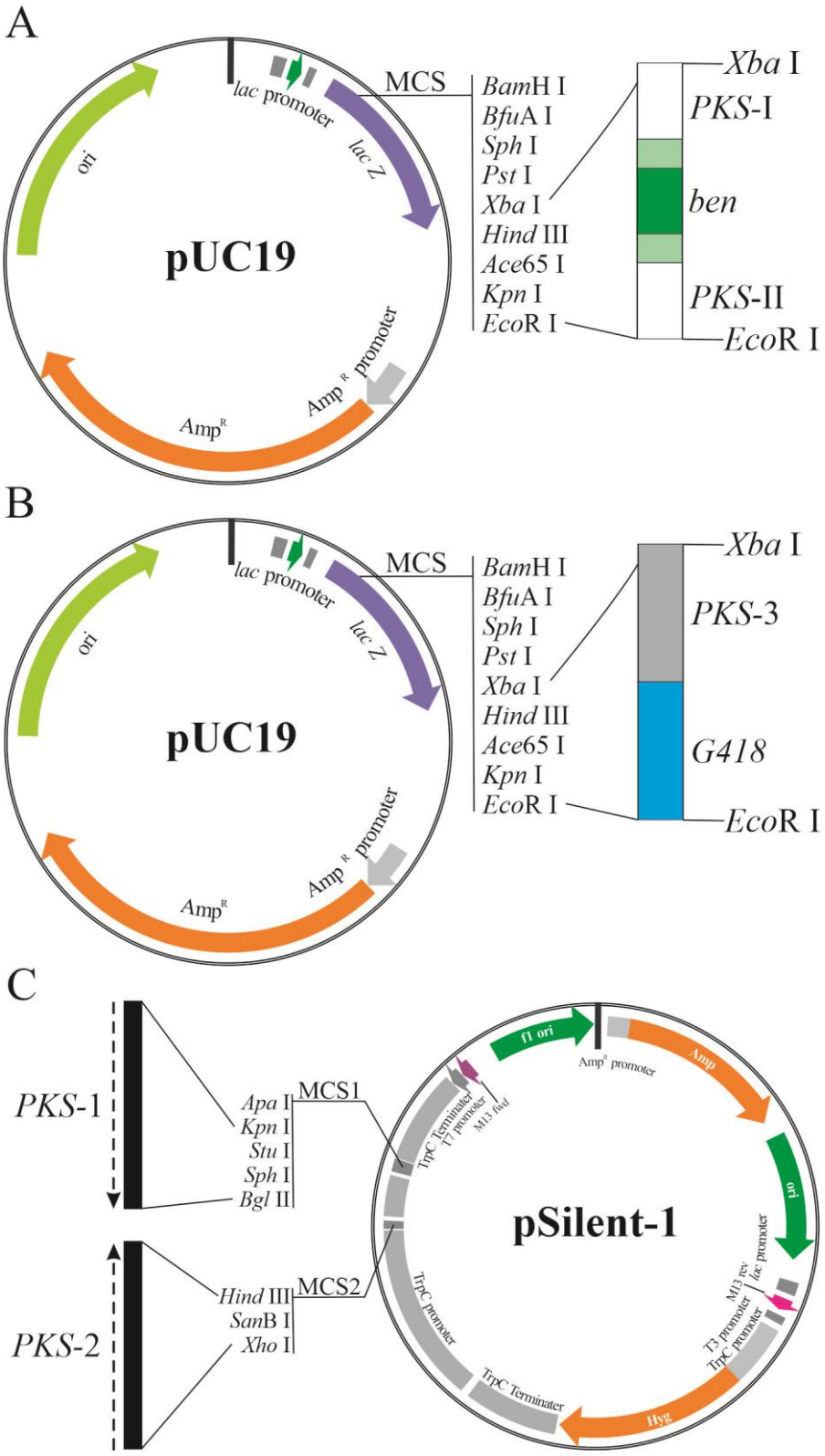
Supplementary Figure 3. RT-qPCR detection of mRNA content of *M. anisopliae* in different time. Error bars represent the standard error of the mean (n = 3).



Supplementary Figure 4. Sensitivity Screening of *M. anisopliae*. A: The sensitivity of *M. anisopliae* to different concentrations of G418; B: The sensitivity of *M. anisopliae* to different concentrations of benomyl; C: Sensitivity of *pks* gene MT of *M. anisopliae* to different concentrations of G418.

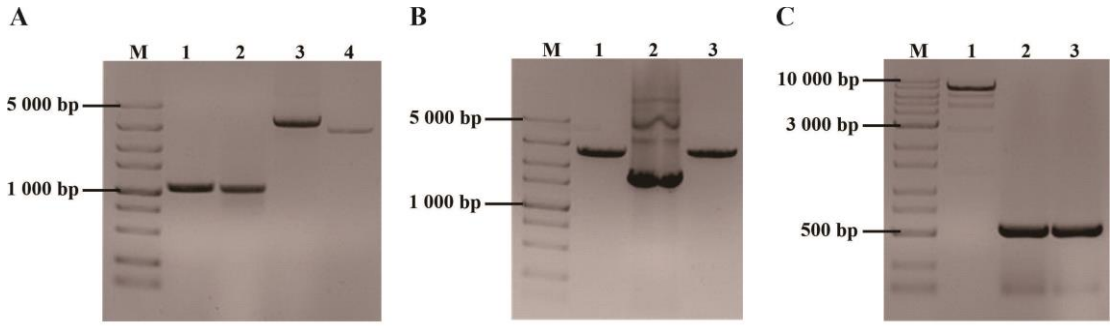


Supplementary Figure 5. Identification figure for *pks* gene MT, CT and RNAi strain.

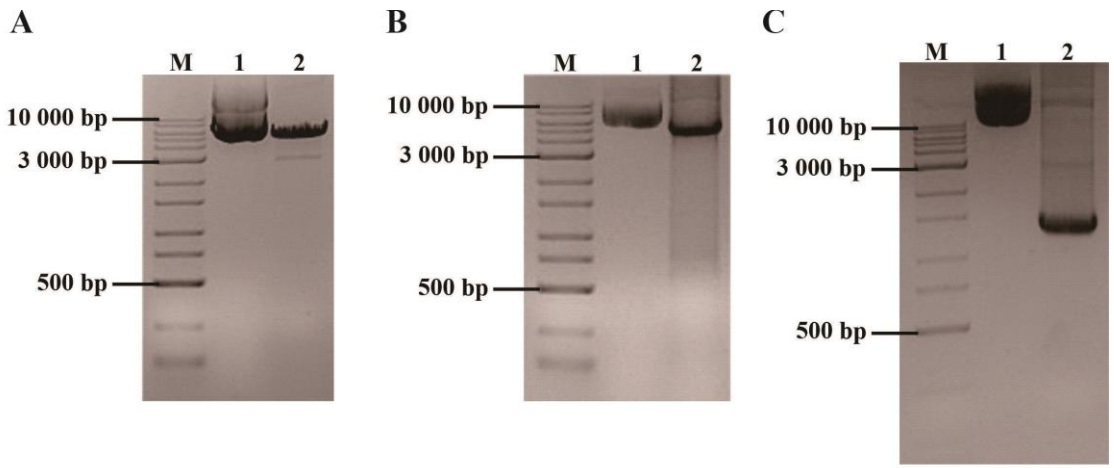


Supplementary Figure 6. Construction of knockout vector, complement vector and RNAi vector of *pks* gene of *M. anisopliae*. A: The upstream (*PKS*-I: 998 bp) and downstream (*PKS*-II: 1,000 bp) fragments of the *pks* gene and the *ben* resistance gene were inserted into the pUC19 vector digested with *Eco*R I/*Bam*H I by In-Fusion® HD Cloning System (Takara) to construct a knockout construct targeting the *pks* gene. B: The *pks* gene fragment (*PKS*-3: 2,500 bp) was amplified using primers L6/R6 from

the genomic DNA of *M. anisopliae*. The *G418* gene was amplified using primers L7/R7 from pSilent-Dual1. To produce a complementation vector, the *pks* gene and *G418* gene were inserted the MCS of pUC19 vector using In-Fusion cloning. C: The *PKS*-1 (500 bp) and *PKS*-2 (500 bp) fragment were amplified using primers L4/R4, L5/R5 respectively from the genomic DNA of *M. anisopliae*. To produce a RNAi vector, the *PKS*-1 and *PKS*-2 fragment were inserted the MCS1 and MCS2 of pSilent-1 vector respectively using In-Fusion cloning.



Supplementary Figure 7. Fragments of *pks* gene mutation vector, complementary vector and RNAi vector of *M. anisopliae*. A: Fragments of *pks* gene mutation vector, lane1: Upstream homology arm of *pks* gene (*PKS*-I: 998 bp), lane 2: Downstream homology arm of *pks* gene (*PKS*-II: 1,000 bp), lane 3: *Ben* gene (3,100 bp), 4: pUC19 plasmid digestion fragment; B: Fragments of *pks* gene complementary vector, lane 1: pUC19 plasmid digestion fragment, lane 2: *G418* gene (1,700 bp), lane 3: Upstream homology arm + *pks* starting sequence + downstream homology arm; C: Fragments of *pks* gene RNAi vector, lane 1: pSilent-1 plasmid digestion fragment, lane 2: *PKS*-1 fragment (500 bp), lane 3: *PKS*-2 fragment (500 bp).



Supplementary Figure 8. *Pks* gene mutation vector, complementary vector and RNAi vector of *M. anisopliae* and its specific fragments. A: *Pks* gene mutation vector and its specific fragments, lane 1: *Pks* gene mutation vector, lane 2: Specific fragments (Upstream homology arm + *ben* gene +Downstream homology arm of *pks* gene) (5,100 bp); B: *Pks* gene complementary vector and its specific fragments, lane 1: *Pks* gene complementary vector; lane 2: Specific fragments (*G418* gene + *PKS*-3) (4,200 bp); C: *Pks* gene RNAi vector and its specific fragments, lane1: *Pks* gene RNAi vector; lane 2: Specific fragments (*PKS*-1 in MCS1 and *PKS*-2 in MCS2) (1,200 bp).

Supplementary Table 1. Identification primers for *PKS* gene MT, CT and RNAi strain

Primers	Products
F8：TGGATTTCAGTTTCGAGGTT	500 bp
R8：ATGAGTGGCATCTCAGATC	
F9：GAATACCATCTCCTTCCAC	580 bp
R9：TGATCTGACCAGTTGCCTA	
F10：CGTCATGCATTGCAGATGA	439 bp
R10：GTGGCAATAATAGCTCAAGG	
F11：CACCTGTCTCCGTTTCC	278 bp
R11：TTTGGTCCTCAACCTCCTT	
F12：CACTGAAGCGGGAAGGGACT	492 bp
R12：GCGGCGATACCGTAAAGCAC	
ITS1：TCCGTAGGTGAACCTGCGG	534 bp
ITS4：TCCTCCGCTTATTGATATGC	

Supplementary Table 2. Primers is used to measure the expression of *PKS* gene

Genes	Primers	Products	GeneBank No.
PKS	F-Q：ACATTGCCCTCCTTTACC R-Q：TGCGCCAGTCACGAACA	245 bp	XM_007826620.1
its (5.8s rDNA)	F-C：AGCGAAATGCGATAAGTAATG R-C：GACGGCTGTGCTGGAAAA	178 bp	NR_132017.1

Supplementary Table 3. Primers for *PKS* gene of *M. anisopliae* to mutation vector, complementary vector and RNAi vector

Target sequence	Template	Primer sequence
PKS	DNA of *M. anisopliae*	F1：AAGTATCATGTTAGTTGCCG R1：GTGCATCCAGGTATAACTA
ben gene	pBARGPE1-EGFP-BenA	Ben-F：aactcgttgtacaactcaagTCAAGAATTAATTCGGTCGA Ben-R：ttaagtggatctcggtgacgCGGGACTTGGCATATG
PKS-I	DNA of *M. anisopliae*	F2：gcctgcaggtcgactctagaGAGCTGAAACCATGGGA R2：tcgaccgaattaattctgaCTTGAGTTGTACAACGAGT
PKS-II	DNA of *M. anisopliae*	F3：ttaagtggatctcggtgacgCGGGACTTGGCATATGTAA R3：aaacgacggccagtgaattcGGCAACGCATCCATTTGAA
PKS-1	DNA of *M. anisopliae*	F4：aacggcagatcttcgcatgcTGGATTTCAGTTTCGAGGTT R4：taccacaggccttagcatgcATGAGTGGCATCTCAGATC
PKS-2	DNA of *M. anisopliae*	F5：tcgataccgtcgacctcgagATGAGTGGCATCTCAGATC R5：aagcttgtacgtaccttcgagTGGATTTCAGTTTCGAGGTT
PKS-3	DNA of *M. anisopliae*	F6：tccgtcaccagccctgggttGAGCTGAAACCATGGGAC R6：aaacgacggccagtgaattcGGCAACGCATCCATTTGAA
G418 gene	pSilent-Dual1	F7：tccgtcaccagccctgggttGAGCTGAAACCATGGGAC R7：aaacgacggccagtgaattcGGCAACGCATCCATTTGAA