Supplementary materials for

AfLaeA, a Global Regulator of Mycelial Growth, Chlamydospore Production, Pathogenicity, Secondary Metabolism, and Energy Metabolism in the Nematode-Trapping Fungus *Arthrobotrys flagrans*

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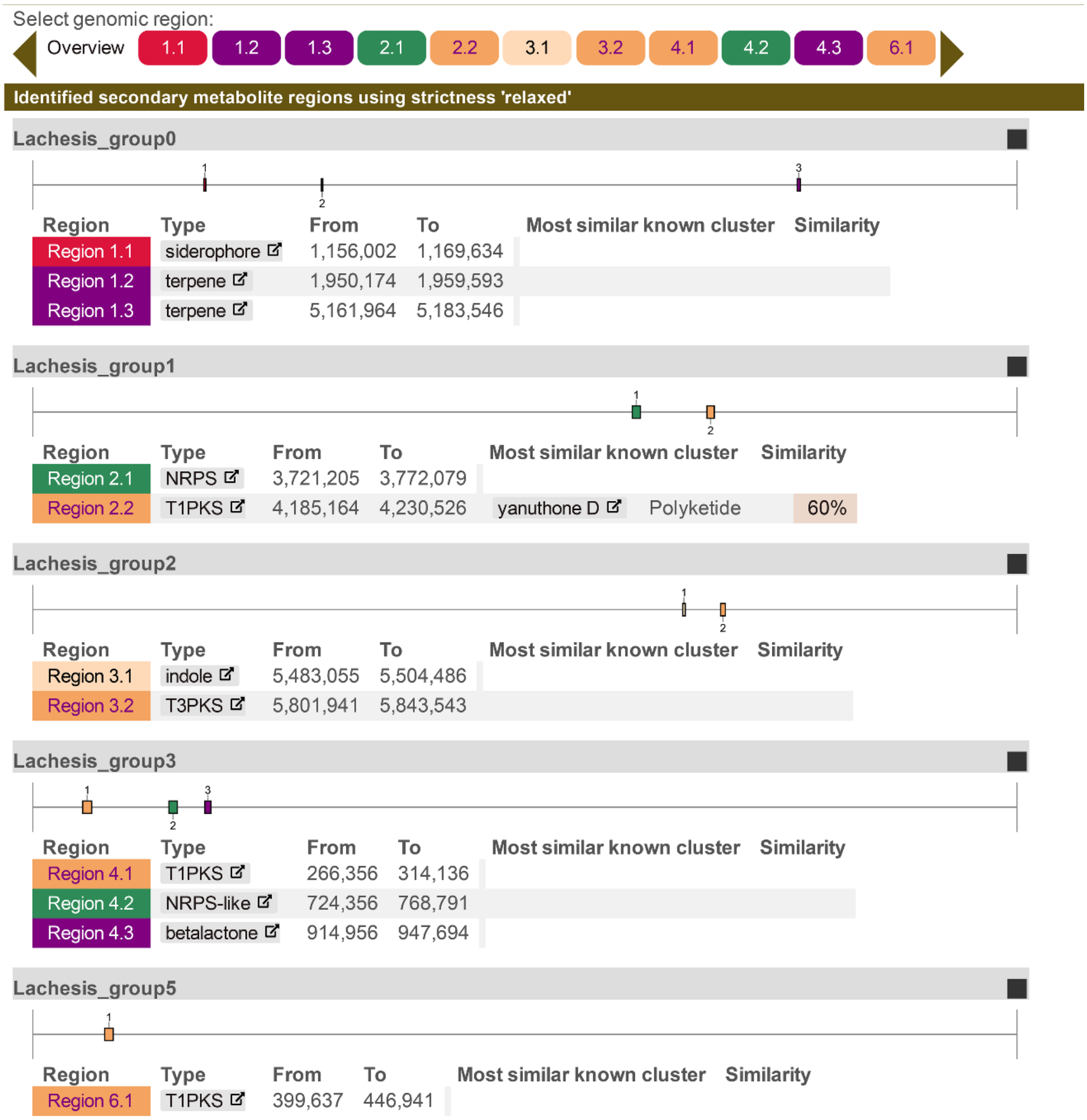
Content



Identified secondary metabolite regions using strictness 'relaxed'

Lachesis_group0

Region	Type	From	To	Most similar known cluster	Similarity
Region 1.1	siderophore	1,156,002	1,169,634		
Region 1.2	terpene	1,950,174	1,959,593		
Region 1.3	terpene	5,161,964	5,183,546		

Lachesis_group1

Region	Type	From	To	Most similar known cluster		Similarity
Region 2.1	NRPS	3,721,205	3,772,079			
Region 2.2	T1PKS	4,185,164	4,230,526	yanuthone D	Polyketide	60%

Lachesis_group2

Region	Type	From	To	Most similar known cluster	Similarity
Region 3.1	indole	5,483,055	5,504,486		
Region 3.2	T3PKS	5,801,941	5,843,543		

Lachesis_group3

Region	Type	From	To	Most similar known cluster	Similarity
Region 4.1	T1PKS	266,356	314,136		
Region 4.2	NRPS-like	724,356	768,791		
Region 4.3	betalactone	914,956	947,694		

Lachesis_group5

Region	Type	From	To	Most similar known cluster	Similarity
Region 6.1	T1PKS	399,637	446,941		

Figure S1 Prediction of secondary metabolites gene clusters in *A. flagrans.*

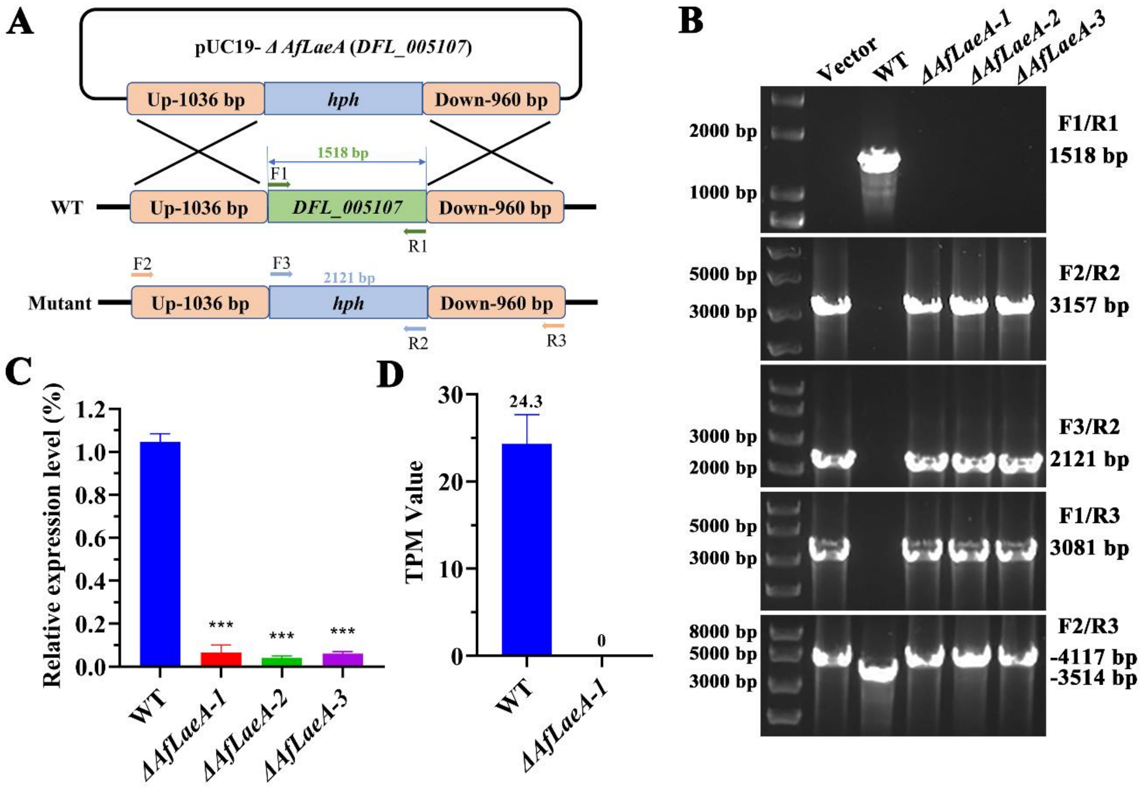


Figure S2 Disruption of *AfLaeA* gene in *A. flagrans.*

A, Diagram of the knockout pattern of the *AfLaeA* gene.

B, Detection of the knockout results of *AfLaeA* gene.

C, Detection of the relative expression levels of *AfLaeA* gene in *ΔAfLaeA* strains by qPCR.

D, Transcriptome datas were used to confirm the expression level *AfLaeA* gene in WT and *ΔAfLaeA* strains.

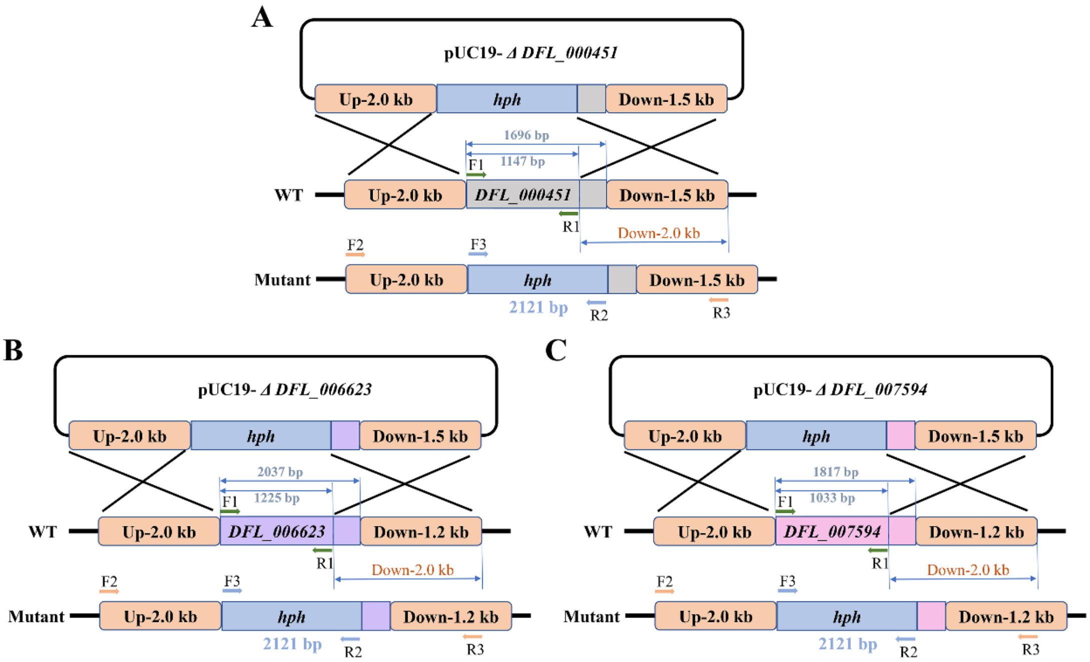


Figure S3 Disruption of three methyltransferases (*DFL_000451*, *DFL_006623*, and *DFL_007594*).

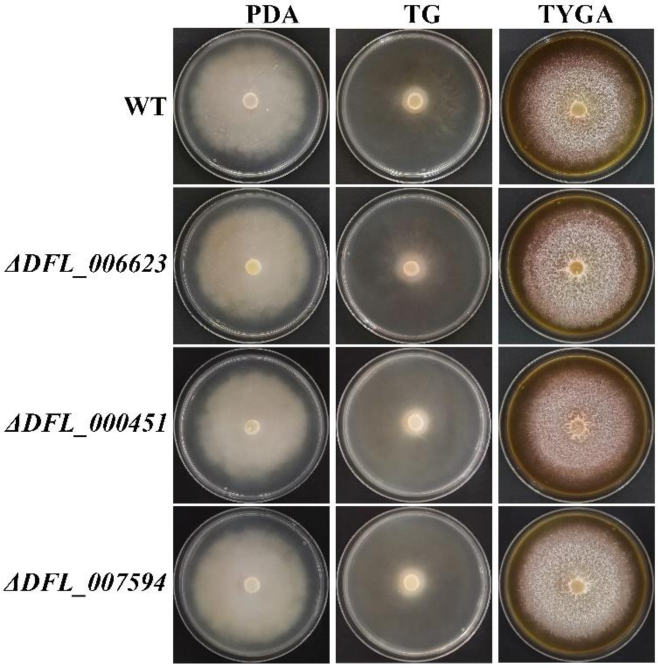


Figure S4 Analysis of the effects of three methyltransferase on mycelium growth. The knock-out strains of *DFL _ 000451*, *DFL _ 006623* and, *DFL _ 007594* genes were cultured with PDA, TG and, TYGA medium at 28℃ for 5 days, respectively.

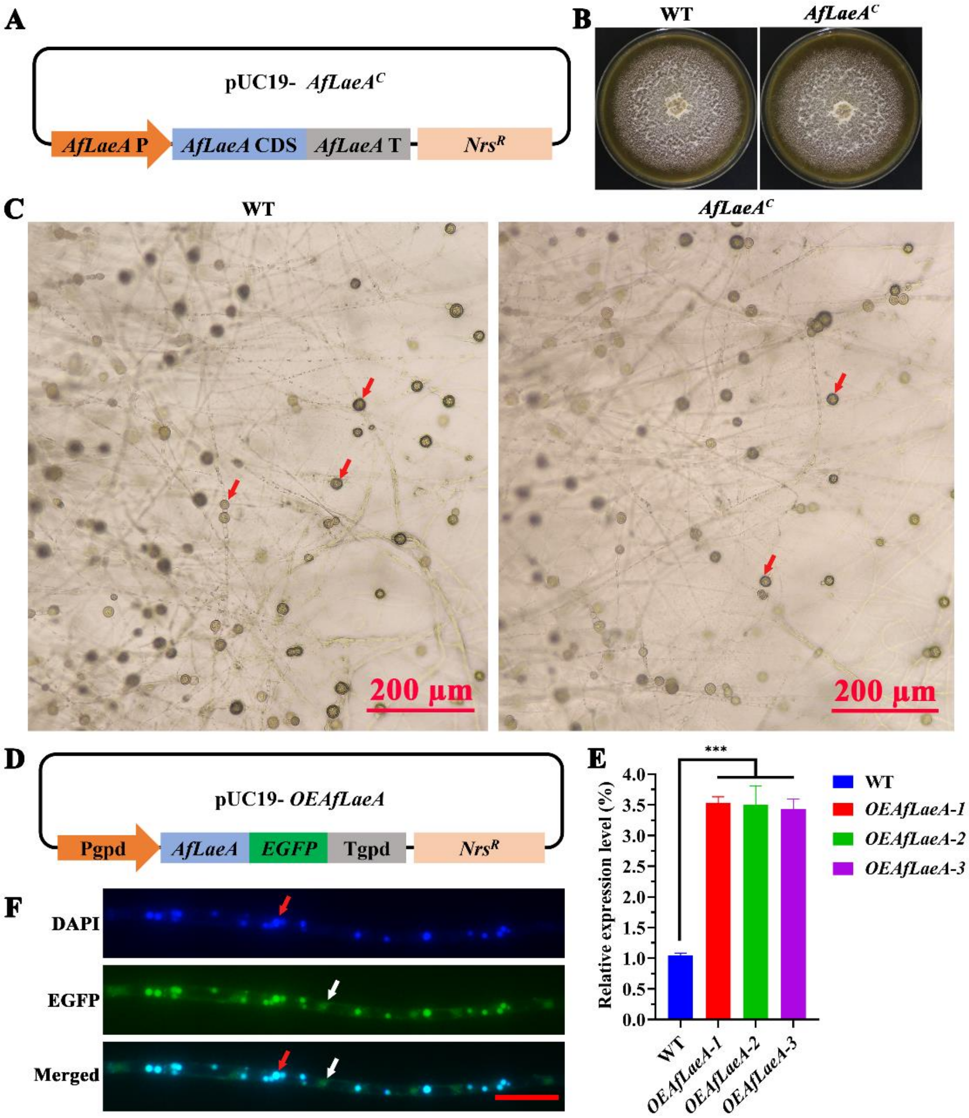


Figure S5 Complementation and overexpression of *AfLaeA* gene in the *ΔAfLaeA*, and WT strain, respectively.

A, Diagram of the complementation pattern of the *AfLaeA* gene by natural promoter in the *ΔAfLaeA* strain.

B, Growth of *A. flagrans* WT, and the complemented strains on TYGA after 7 days.

C, Overview of chlamydospores of the WT and the complemented strains. The red arrows indicate chlamydospores.

D, Diagram of the overexpression pattern of the *AfLaeA* gene.

E, Detection of the relative expression levels of *AfLaeA* gene in *OEAfLaeA* strains by qPCR.

F, Observtion of the localization of AfLaeA in cells by GFP fluorescence. Scale bars, 5 mm.

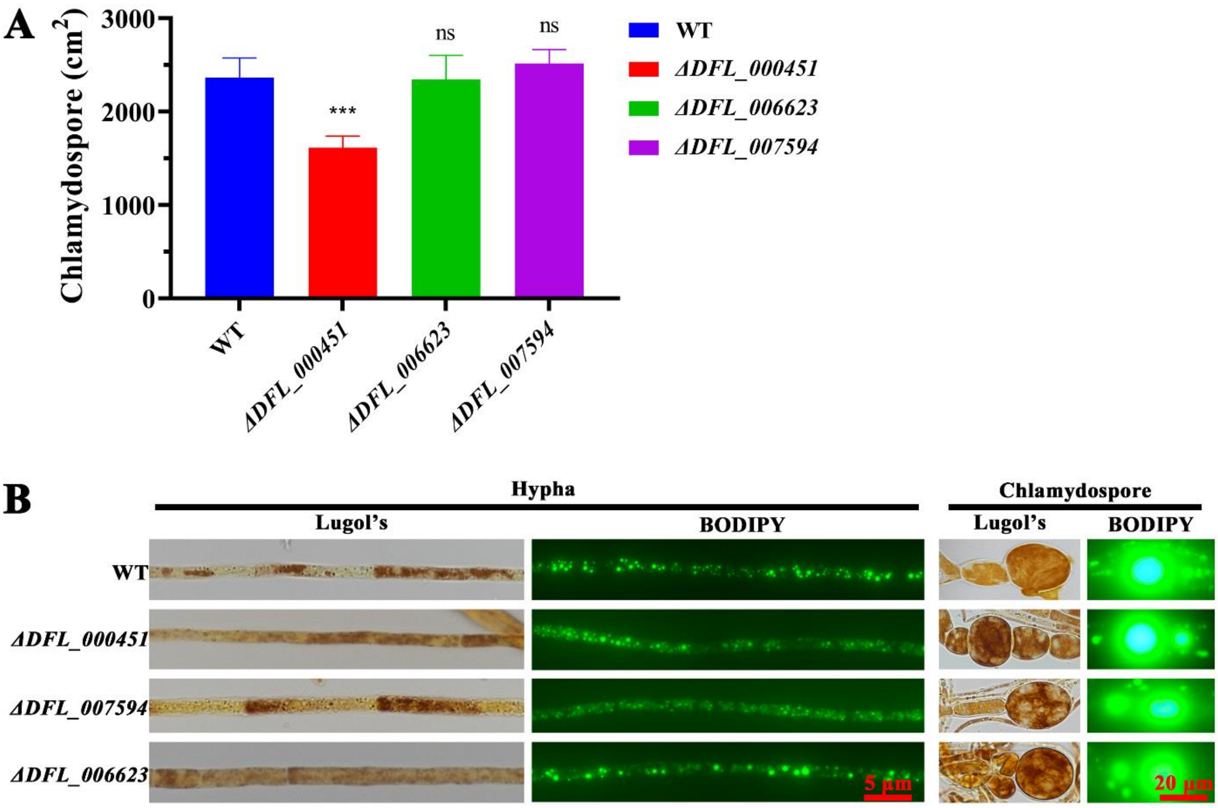


Figure S6 Analysis of the effects of *DFL_000451*, *DFL_006623* and *DFL_007594* genes on chlamydospore, lipid droplets and glycogen production.

A, Comparison of the number of chlamydospores between WT strain and *ΔDFL_000451*, *ΔDFL_006623*. and *ΔDFL_007594* strains (ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

B, Analysis of glycogen and LDs in *ΔDFL_000451*, *ΔDFL_006623*. and *ΔDFL_007594* strains.

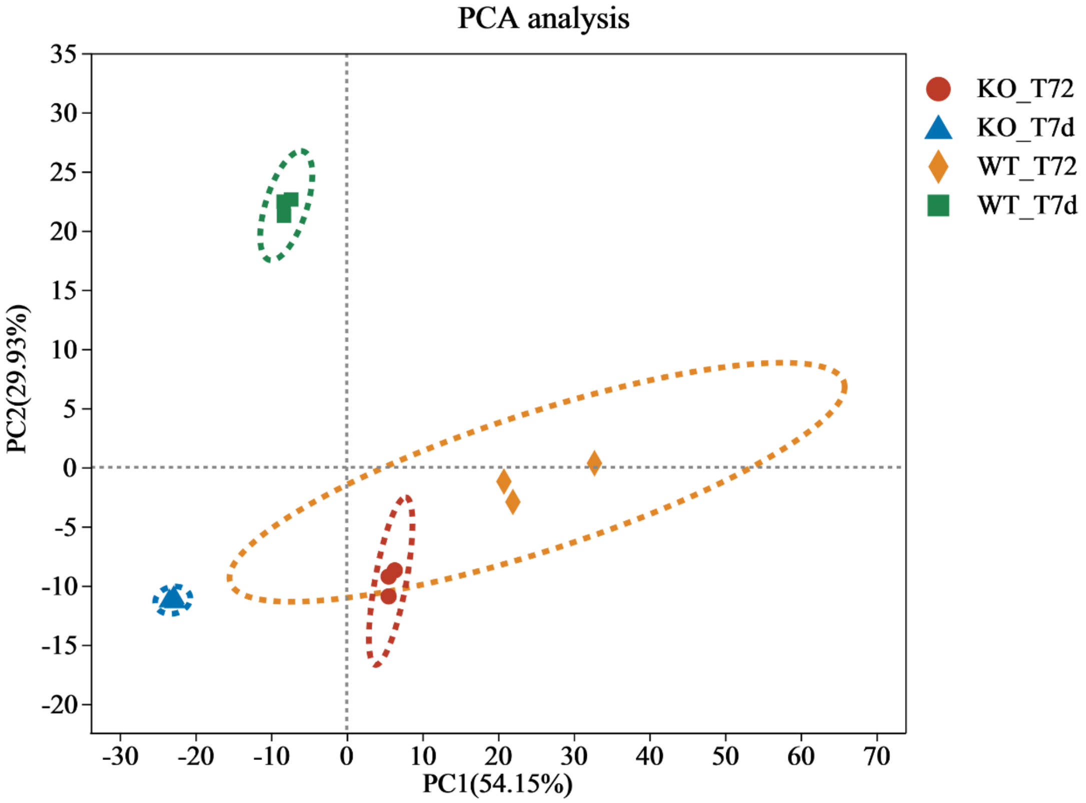


Figure S7 PCA analysis among samples. KO_T72 and WT_T72 represent the *AfLaeA* and WT strains were cultured for 3 days in TYGA medium, respectively. KO_T7d and WT_T7d represent the *ΔfLaeA* and WT strains were cultured for 7 days in TYGA medium, respectively.

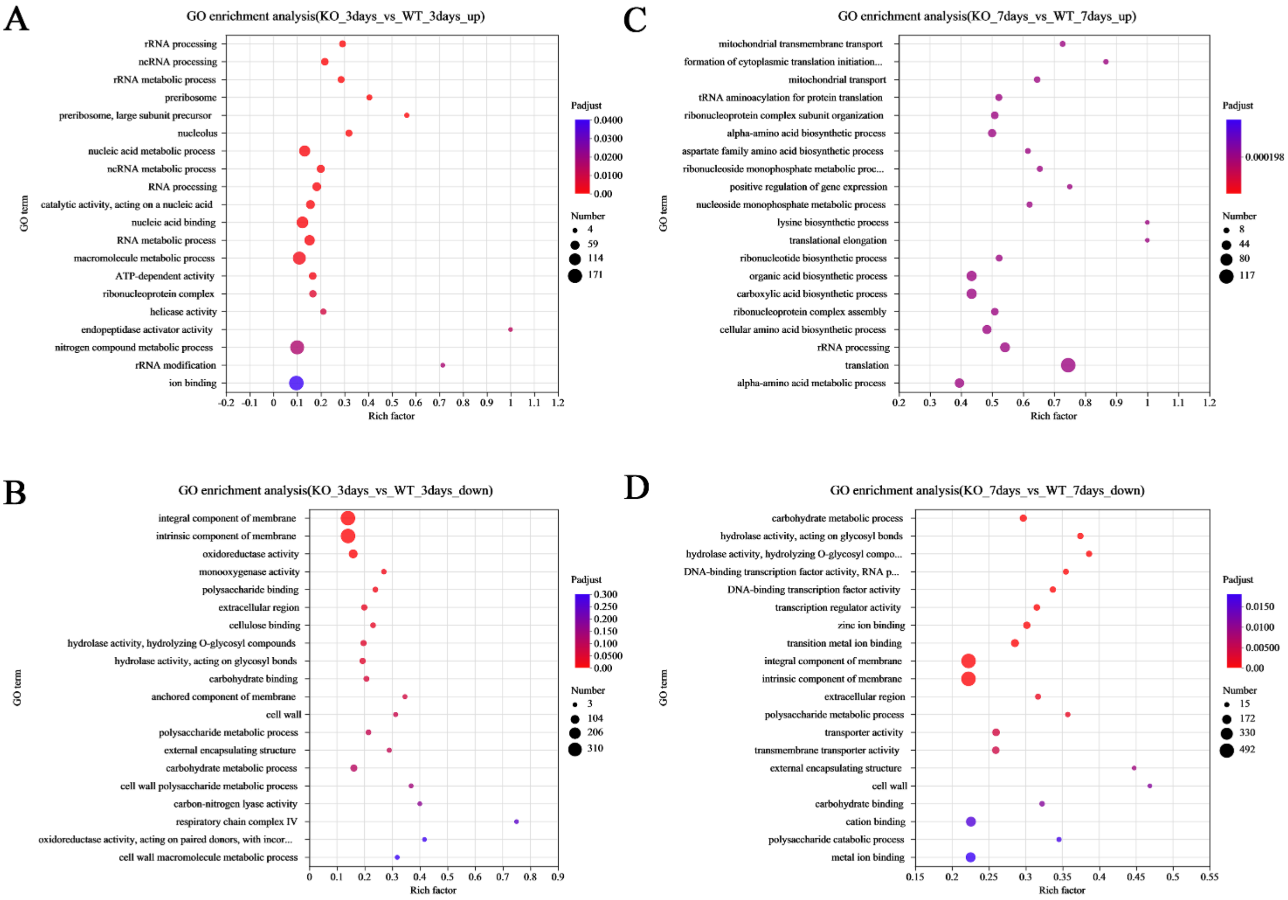


Figure S8 Gene Ontology (GO) enrichment analysis of the DEGs.

A and B, GO enrichment analysis of the upregulated and downregulated DEGs in the *ΔfLaeA* versus WT strain at 3 days.

C and D, GO enrichment analysis of the upregulated and downregulated DEGs in the *ΔfLaeA* versus WT strain at 7 days.

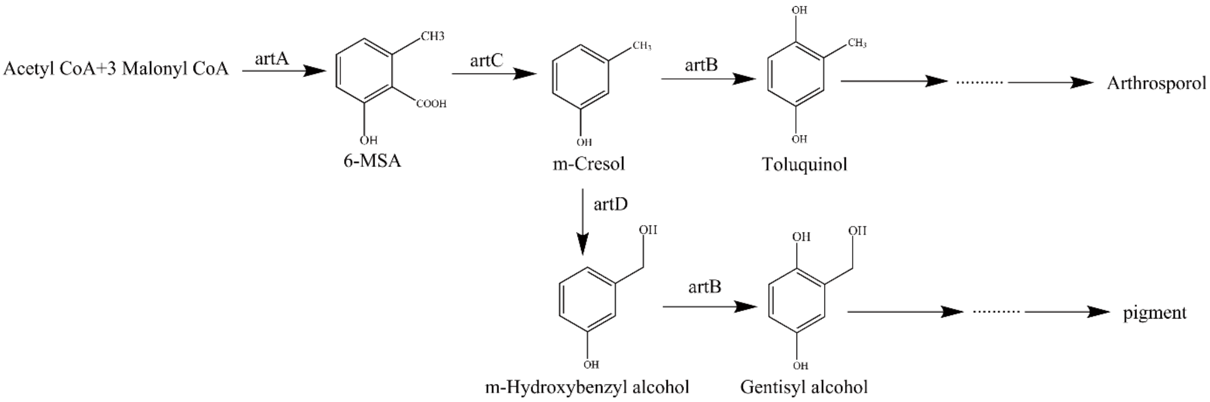


Figure S9 Proposed pathway for the biosynthesis of 6-MSA in *A. flagrans* and *A. oligospora* (1).

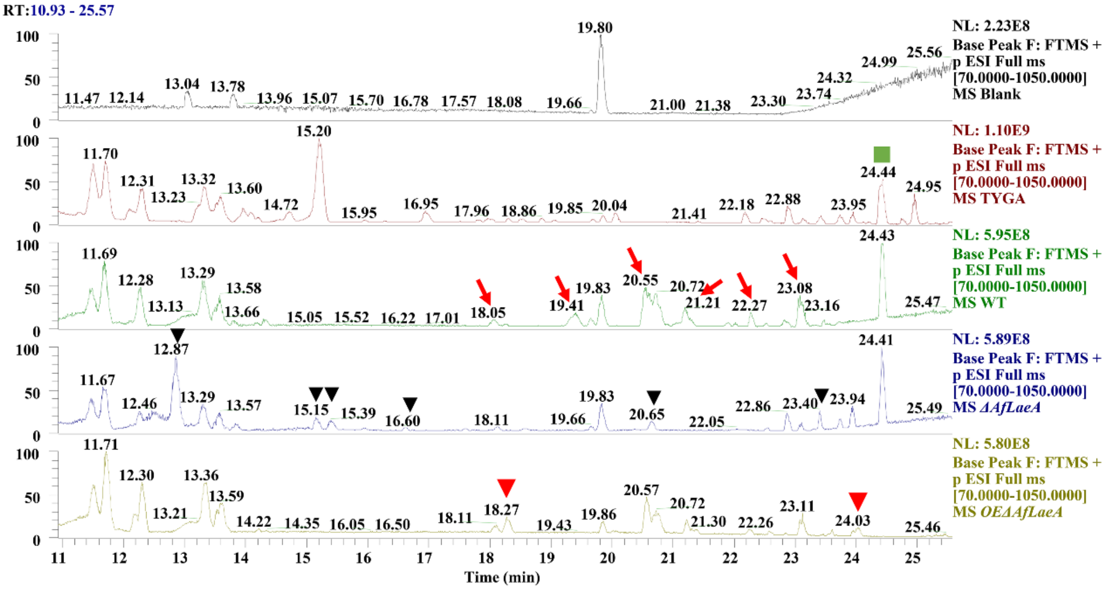


Figure S10 Comparative analysis of the metabolic profile of WT, *ΔAfLaeA*, and *OEAfLaeA* strains cultured in TYGA medium for 14 days by LC-MS. The red arrow indicated the missing components (at m/z 279.2309, 321.2392, 161.0596, and 303.2292 with retention times of 20.55, 21.21, 22.27, and 23.08 min, respectively) of *ΔAfLaeA* strain compared with WT strain. The black and red inverted triangles indicate the newly produced components (at m/z 130.0652, 511.2546, 130.0652, 494.2030, 279.2316, and 379.3354 with retention times of 12.87, 15.15, 15.39, 16.60, 20.65, and 23.40 min, respectively) of *ΔAfLaeA*, and *OEAfLaeA* strains, respectively. Green squares indicate the components with peak time of 24.44 min in TYGA medium, and cannot be used by WT and *ΔAfLaeA* strains.

Table S1 Analysis of the secondary metabolic gene clusters in *A. flagrans*.

#Gene_cluster	scaffold_id	Start	End	Length(bp)
r1c1	Lachesis_group0	1156002	1169634	13633
r1c2	Lachesis_group0	1950174	1959593	9420
r1c3	Lachesis_group0	5161964	5183546	21583
r2c1	Lachesis_group1	3721205	3772079	50875
r2c2	Lachesis_group1	4185164	4230526	45363
r3c1	Lachesis_group2	5483055	5504486	21432
r3c2	Lachesis_group2	5801941	5843543	41603
r4c1	Lachesis_group3	266356	314136	47781
r4c2	Lachesis_group3	724356	768791	44436
r4c3	Lachesis_group3	914956	947694	32739
r6c1	Lachesis_group5	399637	446941	47305

Table S2 Comparison of the conserved domain of methyltransferase between *A. nidulans* and *A. flagrans* by NCBI online tool Conserved Domain Search Service.

Protein					Domain Name	Description
AnLaeA (AAQ95166.1)	AfLaeA (DFL_005107) (EVM02G003030)	DFL_000451 (EVM05G004610)	DFL_006623 (EVM03G001000)	DFL_007594 (EVM03G010660)		
◆	●	▲	★	■	Methyltransf_25	Methyltransferase domain
◆	●	▲	★	■	Methyltransf_23	Methyltransferase domain
◆	●	▲	★	■	AdoMet_MTases	S-adenosylmethionine-dependent methyltransferases
◆	●	▲		■	Methyltransf_12	Methyltransferase domain
◆	●	▲	★	■	Methyltransf_11	Methyltransferase domain
	●	▲	★	■	PLN02336	phosphoethanolamine N-methyltransferase
◆	●	▲	★	■	HemK	Methylase of polypeptide chain release factors
◆	●	▲	★	■	Methyltransf_31	Methyltransferase domain
	●			■	Cfa	Cyclopropane fatty-acyl-phospholipid synthase and related methyltransferases
	●		★		PRK09328	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase
◆	●	▲	★	■	UbiE	Ubiquinone/menaquinone biosynthesis C-methylase
	●	▲	★	■	BioC	malonyl-acyl carrier protein O-methyltransferase
	●			■	PRK14968	putative methyltransferase
◆	●	▲	★	■	TrmN6	tRNA1(Val) A37 N6-methylase
	●	▲		■	ubiE	bifunctional demethylmenaquinone methyltransferase/2-methoxy-6-polyprenyl-1,4-benzoquinol methylase
	●		★	■	hemK_fam	HemK family putative methylases
	●	▲	★		MenG_MenH_UbiE	ubiquinone/menaquinone biosynthesis methyltransferases

	●				Methyltransf_16	Lysine methyltransferase
	●				PRK01544	bifunctional N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase/tRNA (m7G46)
◆	●			■	MTS	Methyltransferase small domain
		▲	★	■	Tam	Trans-aconitate methyltransferase
		▲	★	■	Ubie_methyltran	ubiE/COQ5 methyltransferase family
		▲			PLN02244	tocopherol O-methyltransferase
		▲	★	■	SmtA	SAM-dependent methyltransferase
		▲			PTZ00098	phosphoethanolamine N-methyltransferase
◆		▲		■	PRK09328	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase
			★		PLN02490	MPBQ/MSBQ methyltransferase
			★		CMAS	Mycolic acid cyclopropane synthetase
			★	■	PRK07580	Mg-protoporphyrin IX methyl transferase
			★		PKS_MT	Methyltransferase in polyketide synthase (PKS) enzymes
			★		PRK01683	trans-aconitate 2-methyltransferase
				■	YrrM	Predicted O-methyltransferase YrrM
◆				■	RsmC	16S rRNA G1207 methylase RsmC
				■	PRK14103	trans-aconitate 2-methyltransferase
◆					FtsJ	FtsJ-like methyltransferase

Table S3 BLAST search of the hypothetical LaeA using AnLaeA (GeneBank: AAQ95166.1) as a query sequence in *A. flagrans* genome.

Gene ID	GeneBank	Percent Identity	Query Cover	E value
DFL_005107	RVD86853.1	48.47%	76%	6e-96
DFL_000451	RVD89443.1	37.15%	76%	1e-57
DFL_006623	RVD82190.1	37.92%	78%	5e-55
DFL_007594	RVD83196.1	35.26%	81%	2e-53
DFL_006144	RVD84392.1	31.76%	84%	2e-51
DFL_006658	RVD82225.1	34.00%	78%	4e-48
DFL_006575	RVD82141.1	33.77%	78%	1e-47
DFL_002107	RVD87904.1	31.80%	81%	4e-45
DFL_003019	RVD88847.1	27.57%	71%	7e-30
DFL_001934	RVD87721.1	25.29%	43%	3e-08

Table S4 The predicted and tallied physiochemical properties of AfLaeA, and three methyltransferase in *A. flagrans*.

Gene Name	Exon Number	Intron Number	Number of Amino Acids (aa)	Molecular Weight (kDa)	Subcellular localization	Reference
AnLaeA	-	-	374	43.0	Nuclear, Cytoplasmic	(2)
AfLaeA	7	6	362	41.491	Nuclear, Cytoplasmic	This study
DFL_000451	11	10	340	38.530	Cytoplasmic	This study
DFL_006623	12	11	367	40.620	Cytoplasmic	This study
DFL_007594	9	8	353	40.659	Cytoplasmic	This study

Table S5. Statistics of reads and mapping rate for the WT strain and *ΔAfLaeA* (KO) strains at different time points.

Sample	Total reads	Total mapped	Multiple mapped	Uniquely mapped
KO_T3d_3	43429426	41931260(96.55%)	718954(1.66%)	41212306(94.89%)
KO_T3d_2	42592746	40677347(95.5%)	788589(1.85%)	39888758(93.65%)
KO_T3d_1	50354928	48246991(95.81%)	905301(1.8%)	47341690(94.02%)
WT_T3d_3	48099230	45830147(95.28%)	1247476(2.59%)	44582671(92.69%)
WT_T3d_2	42921210	41080883(95.71%)	1016245(2.37%)	40064638(93.34%)
WT_T3d_1	56412094	53881198(95.51%)	1480983(2.63%)	52400215(92.89%)
KO_T7d_3	62833816	60393778(96.12%)	1243743(1.98%)	59150035(94.14%)
KO_T7d_2	60455376	58187374(96.25%)	1030325(1.7%)	57157049(94.54%)
KO_T7d_1	59156000	57426141(97.08%)	1080771(1.83%)	56345370(95.25%)
WT_T7d_3	64070156	61837821(96.52%)	1529355(2.39%)	60308466(94.13%)
WT_T7d_2	48599186	46912912(96.53%)	1095932(2.26%)	45816980(94.28%)
WT_T7d_1	50418794	48597415(96.39%)	1182796(2.35%)	47414619(94.04%)

Table S6 Relative content of unknown peaks in *ΔAfLaeA* and *ΔAfLaeA*^{RC} strains compared with WT (Top 20).

ΔAfLaeA					*ΔAfLaeA*^{RC}				
Predict molecular formula	Calc. MW	m/z	RT [min]	Ratio *ΔAfLaeA* /WT	Predict molecular formula	Calc. MW	m/z	RT [min]	Ratio *ΔAfLaeA*^{RC} /WT
$C_{20}H_{30}N_2O_2$	330.23056	331.23784	17.007	0.001771287	$C_{28}H_{36}N_4O$	444.2885	445.2958	17.186	1204.756
$C_{20}H_{32}N_2O$	316.25142	317.25869	16.951	0.002223715	$C_{28}H_{36}N_4O$	444.2887	445.2959	17.095	918.1334
$C_{10}H_{11}NOS$	193.05616	194.06344	16.929	0.003421061	$C_{20}H_{26}N_6O_4$	414.2022	415.2095	14.138	808.3236
$C_8H_{11}N_3O_3$	197.08009	198.08737	16.677	0.005762016	$C_{28}H_{36}N_4O$	444.2886	445.2959	15.75	588.2408
$C_{17}H_{14}O_7$	330.07386	331.08114	15.558	0.006608889	$C_9H_{23}N_4O_3P$	266.1498	267.157	12.46	558.5093
$C_{18}H_{15}N_3O$	289.12098	290.12826	16.977	0.007809357	$C_8H_{10}N_8O_2S$	282.0656	283.0728	0.522	549.7278
$C_{12}H_{27}NO$	201.20934	202.21662	15.651	0.008059129	$C_{20}H_{34}N_2O_3$	350.2568	351.264	11.036	487.2139
$C_{16}H_{10}O_6$	298.04755	299.05483	16.091	0.008960139	$C_{16}H_{27}N_4OP$	322.1939	323.2012	15.686	316.4279
$C_{16}H_{17}N_3$	251.14197	252.14925	15.65	0.009700595	$C_{14}H_{33}N_6O_2P$	348.2412	349.2484	11.677	316.3023
$C_{10}H_{11}NO_2S_2$	241.02307	242.03035	15.739	0.010699206	$C_4H_6N_2O$	98.04851	99.05572	13.616	314.4503
$C_{19}H_{30}N_2O$	302.2364	303.24368	16.944	0.01240193	$C_7H_6N_6OS$	222.0329	223.0402	10.416	294.5013
$C_{14}H_{32}N_6O_3S$	364.22448	365.2316	16.916	0.013988137	$C_{18}H_{33}N_4O_4P$	400.2231	401.2303	19.291	271.6765
$C_6H_5N_7O$	191.05591	192.06319	3.362	0.014213441	$C_{14}H_{33}N_6OP$	332.2461	333.2534	0.678	271.3978
$C_{28}H_{28}N_{10}O_{11}$	680.19516	681.20243	15.555	0.014471258	$C_{20}H_{34}N_2O_3$	350.2567	351.2639	0.85	269.5328
$C_9H_{21}N_4OP$	232.14446	233.15174	15.778	0.016945244	$C_6H_{19}N_7OS$	237.1364	238.1437	0.667	258.3884
$C_{20}H_{28}O_3$	316.2039	317.21128	18.564	0.017027088	$C_{11}H_{21}N_4O_2P$	272.1391	273.1464	17.081	255.7101
$C_{14}H_{31}NO_3$	261.2303	262.23757	16.101	0.017126961	$C_{11}H_{17}N_6O_2P$	296.116	297.1233	10.603	246.8178
$C_{20}H_{14}N_2O_2$	314.10519	315.11247	16.191	0.017209785	$C_4H_6N_2O$	98.04852	99.05572	13.841	245.8484545
$C_{11}H_{13}N_6OP$	276.0897	277.09698	16.83	0.018492191	$C_{20}H_{28}N_3P_3$	403.1507	404.1579	10.403	239.3218023
$C_{23}H_{35}N_3O_3$	401.26772	402.275	16.672	0.019293874	$C_{20}H_{34}N_2O_3$	350.2567	351.2641	0.638	221.8894215

Table S7 Presence and effects of *LaeA* in the fungal kingdom.

Fungal groups	Species	Function					Reference
		SM	G	D	V	SP	
Ascomycota	*Aspergillus nidulans*	SM	-	D	-	-	(3, 4)
	Aspergillus fumigatus	SM	-	D	V	-	(5, 6)
	Aspergillus oryzae	SM	-	D	-	-	(7, 8)
	Aspergillus flavus	SM	-	D	V	SP	(9, 10)
	Aspergillus carbonarius	SM	-	D	V	-	(11)
	Aspergillus luchuensis	SM	-	D	-	-	(12)
	Aspergillus niger	SM	G	D	-	-	(13, 14)
	Aspergillus terreus	SM	-	D	-	-	(15, 16)
	Aspergillus ochraceus	SM	-	D	V	-	(17)
	Aspergillus versicolor	SM	-	D	-	-	(18)
	Aspergillus pachycristatus	SM	-	D	-	-	(19)
	Aspergillus pseudoterreus	SM	-	D	-	-	(20)
	Aspergillus fumisynnematus	SM	-	D	-	-	(21)
	Monascus pilosus	SM	-	D	-	-	(22)
	Monascus ruber	SM	G	D	-	-	(23)
	Monascus purpureus	SM	-	D	-	-	(24)
	Trichoderma atroviride	SM	G	D	-	-	(25)
	Trichoderma reesei	SM	G	D		SP	(26, 27)
	Trichoderma afroharzianum	SM	-	-	-	-	(28)
	Trichoderma longibrachiatum SMF2	SM	G	D	-	SP	(29)
	Penicillium Brocae HDN-12-143	SM	-	-	-	-	(30)
	Penicillium dipodomyis YJ-11	SM	-	-	-	-	(31)
	Penicillium digitatum	SM	-	D	-	-	(32)
	Penicillium chrysogenum	SM	G	D	-	-	(33, 34)
	Penicillium citrinum	SM	-	D	-	-	(35, 36)
	Penicillium expansum	SM	G	D	V	-	(37, 38)
	Penicillium oxalicum	SM	-	D	-	-	(39)
	Fusarium verticillioides	SM	-	D	V	-	(40)
	Fusarium graminearum	SM	G	D	V	-	(41)
	Fusarium oxysporum	SM	G	D	V	-	(42)
	Fusarium fujikuroi	SM	-	D	V	-	(43)
	Cladosporium fulvum	SM	G	D	V	-	(44)
	Beauveria bassiana	SM	-	-	V	SP	(45)
	Cordyceps militaris	SM	-	-	-	-	(46)
	Botrytis cinerea	SM	-	D	V	SP	(47)
	Pestalotiopsis microspora	SM	G	D	V	-	(48)
	Valsa mali	SM	G	D	V	-	(49)
	Cochliobolus heterostrophus	SM	G	D	V	-	(50)
	Neurospora crassa	SM	G	D	V	-	(51)
	Chaetomium globosum	SM	G	D	V	-	(52)
	Dothistroma septosporum	SM	G	D	V	-	(53)
	Alternaria alternata	SM	G	D	V	-	(54, 55)
	Alternaria alstroemeria	SM	-	-	V	-	(56)
	Epichloe festucae	SM	G	D	V	SP	(57, 58)
	Magnaporthe oryzae	SM	-	D	V	-	(59)
	Pyricularia oryzae	SM	-	-	-	-	(60)
Basidiomycete	*Pleurotus ostreatus*	SM	G	D	-	SP	(61)
	Ganoderma lingzhi	SM	G	D	-	-	(62)
	Coprinopsis cinerea	SM	G	D	-	-	(63)

SM: secondary metabolite; G: Growth; D: fungal development; V: virulence; SP: secreted protein

Table S8 PCR primer sets used in this study.

Target gene	Sequence ID	Primers	Primer sequences (5'-3') [a]	Uses
AfLaeA	DFL_005107 (EVM02G003030)	Ko5107-up-for	ccgggtaccgagctcgaattcTTCGTTACCTTTTTTGCTTATTCCT	Amplify the 5’ flank of *AfLaeA* gene
		Ko5107-up-rev	cttctgTCAGACCAATTCCCCGGAA	
		Ko5107-hyg-for	gggaattggtctgaCAGAAGATGATATTGAAGGAGCATTT	Amplify the *hph* cassette
		Ko5107-hyg-rev	attgAAAGAAGGATTACCTCTAAACAAGTGTACC	
		Ko5107-down-for	gaggtaatccttctttCAATTATTAACAACTACGGCTCGC	Amplify the 3’ flank of *AfLaeA* gene
		Ko5107-down-rev	gtactgagagtgcaccatatgTTGGAAAGGTGGGGAATTAGG	
DFL_000451	DFL_000451 (EVM05G004610)	Ko0451-up-for	ccgggtaccgagctcgaattcCCCCAACGGTTCCTTTTGC	Amplify the 5’ flank of *DFL_000451* gene
		Ko0451-up-rev	catcttctgTGTGTCCAGTGCGGTTTGTCG	
		Ko0451-hyg-for	cactggacacaCAGAAGATGATATTGAAGGAGCATTT	Amplify the *hph* cassette
		Ko0451-hyg-rev	ccaggaggataAAAGAAGGATTACCTCTAAACAAGTGTACC	
		Ko0451-down-for	tccttctttTATCCTCCTGGTATCATTCAATATTAATAA	Amplify the 3’ flank of *DFL_000451* gene
		Ko0451-down-rev	gtactgagagtgcaccatatgTATGGTGCAAAGTAAATTGGTTTGA	
DFL_006623	DFL_006623 (EVM03G001000)	Ko6623-up-for	ccgggtaccgagctcgaattcTTTTGCGGTATATCTACAAATTCTAGTATAT	Amplify the 5’ flank of *DFL_006623* gene
		Ko6623-up-rev	tcatcttctgGGCTATAGACTTTTTTCTGGGGC	
		Ko6623-hyg-for	gtctatagccCAGAAGATGATATTGAAGGAGCATTT	Amplify the *hph* cassette
		Ko6623-hyg-rev	ccctatcatcggagaaaagtAAAGAAGGATTACCTCTAAACAAGTGTACC	
		Ko6623-down-for	ACTTTTCTCCGATGATAGGGTTCC	Amplify the 3’ flank of *DFL_006623* gene
		Ko6623-down-rev	gtactgagagtgcaccatatgCCAACTTCAAAGGAAAAGGATTAAA	
DFL_007594	DFL_007594 (EVM03G010660)	Ko7594-up-for	ccgggtaccgagctcgaattcGCTTCTTTTCGACTGTTGAAACC	Amplify the 5’ flank of *DFL_007594* gene
		Ko7594-up-rev	tctgCGATTTTAGAATAGGGAAAACCCT	
		Ko7594-hyg-for	ccctattctaaaatcgCAGAAGATGATATTGAAGGAGCATTT	Amplify the *hph* cassette
		Ko7594-hyg-rev	ggaaggataatggagagactAAAGAAGGATTACCTCTAAACAAGTGTACC	
		Ko7594-down-for	AGTCTCTCCATTATCCTTCCAAATCA	Amplify the 3’ flank of *DFL_007594* gene
		Ko7594-down-rev	gtactgagagtgcaccatatgGAAAATCGCACCGGGTAGTTG	
AfLaeA	DFL_005107 (EVM02G003030)	RC-5107-gpdp-for	ccgggtaccgagctcgaattcGATCACCTCGGCGGGGTC	Amplify the promoter of *Afgpd* gene
		RC-5107-gpdp-rev	ggtagacttctgtcatTTTGAATTATTGACTTTTGTCGAGG	
		RC-5107-cds-for	caaaATGACAGAAGTCTACCAATACGCTCA	Amplify the CDS fragment of *AfLaeA* gene
		RC-5107-cds-rev	ttgctcaccatAGGGCGTCGGGCAATCCA	
		RC-5107-egfp-for	cgacgccctATGGTGAGCAAGGGCGAGG	Amplify the *EGFP* fragment
		RC-5107-egfp-rev	gcttcacgctggggaattatTTACTTGTACAGCTCGTCCATGCC	
		RC-5107-gpdT-for	ATAATTCCCCAGCGTGAAGCT	Amplify the terminator of *Afgpd* gene
		RC-5107-gpdT-rev	gcctccatgtcATGTGGAAAAGGGATTTGATCG	
		RC-5107-nrsr-for	tttccacatGACATGGAGGCCCAGAATACC	Amplify the Nrs^R cassette

		RC-5107-nrsr-rev	gtactgagagtgcaccatatgCAGTATAGCGACCAGCATTCACA	
AfvosA	DFL_008845 (EVM00G008390)	AD-8390-for	gagtggccattatggATGCAAAATCATTCCTTTGCAGA	Amplify the CDS fragment of *AfveA* gene
		AD-8390-rev	gccgacatgttttttTCATGTAGCATAAGTTTGGCTAACAG	
AfveA	DFL_000190 (EVM05G002000)	AD-5070-for	gagtggccattatggATGGCCGACCGTTTTATCCC	Amplify the CDS fragment of *AfveA* gene
		AD-5070-rev	gccgacatgttttttTTACATGTCCATAGTATCACCCTGG	
AfvelB	DFL_008938 (EVM00G009330)	AD-9330-for	gagtggccattatggATGTATACTACAAGCTCTCGGGACC	Amplify the CDS fragment of *AfvelB* gene
		AD-9330-rev	gccgacatgttttttTCAGTAATCGTCATCATCGTCCTG	
AfvelC	DFL_004053 (EVM02G013760)	AD-3760-for	gagtggccattatggATGATGTCTCCTAGGCCTCCACC	Amplify the CDS fragment of *AfvelC* gene
		AD-3760-rev	gccgacatgttttttTTATGCTTTCCTGCGACGTTT	
AfpacC	DFL_007387 (EVM03G008580)	AD-8580-for	gagtggccattatggATGGATAGCACGGAACCACG	Amplify the CDS fragment of *AfpacC* gene
		AD-8580-rev	gccgacatgttttttTTATGCGGATGTTTCGACAGAC	
AfbrlA	DFL_001688 (EVM01G002650)	AD-2650-for	gagtggccattatggATGTCCCATCATAACATTCACATGG	Amplify the CDS fragment of *AfbrlA* gene
		AD-2650-rev	gccgacatgttttttTCAATGCTCAAGCTTGATCCC	
AfkapA	DFL_007032 (EVM03G005070)	AD-7032-for	catggaggccgaattcccgggATGGCCGACCGTTTTATCCC	Amplify the CDS fragment of *AfkapA* gene
		AD-7032-rev	gcaggtcgacggatccccgggTTACATGTCCATAGTATCACCCTGG	
AffphA	DFL_002824 (EVM01G014010)	AD-4010-for	gagtggccattatggATGGAAATCTTCAATATTTTGTCACAA	Amplify the CDS fragment of *AffphA* gene
		AD-4010-rev	gccgacatgttttttTCACCCTATGTGTGTCTGGAAGG	
AfLaeA	DFL_005107 (EVM02G003030)	AD-5107-for	catggaggccgaattcccgggATGACAGAAGTCTACCAATACGCTCA	Amplify the CDS fragment of *AfLaeA* gene
		AD-5107-rev	gcaggtcgacggatccccgggTTAAGGGCGTCGGGCAAT	
		BD-5107-for	catggaggccgaattcccgggATGACAGAAGTCTACCAATACGCTCA	
		BD-5107-rev	gcaggtcgacggatccccgggTTAAGGGCGTCGGGCAAT	
AfLaeA	DFL_005107 (EVM02G003030)	RC-5107-P-for	ccgggtaccgagctcgaattcGTTTCTATCATGAGCATGTATCCATG	Amplify the 5’ flank (2 kb) of *AfLaeA* gene
		RC-5107-P-rev	gtcatTCAGACCAATTCCCCGGAA	
		RC-5107-CDS-for	ggggaattggtctgaATGACAGAAGTCTACCAATACGCTCA	Amplify the CDS fragment of *AfLaeA* gene
		RC-5107-CDS-rev	taattgTTAAGGGCGTCGGGCAAT	
		RC-5107-T-for	cccgacgcccttaaCAATTATTAACAACTACGGCTCGC	Amplify the 3’ flank (1 kb) of *AfLaeA* gene
		RC-5107-T-rev	gcctccatgtcGAAGGAAAAGGGTGAAATTTGTCT	
		RC-5107-NRS-for	ttttccttcGACATGGAGGCCCAGAATACC	Amplify the *NRS*^{R} cassette
		RC-5107-NRS-rev	gtactgagagtgcaccatatgCAGTATAGCGACCAGCATTCACA	

[a] The primer sequence consistent with the vector is indicated by lowercase letters.

Table S9 Real time PCR primer sets used in this study.

Target gene	Sequence ID	Primers	Primer sequences (5'-3')
AfGpd	DFL_000198 (EVM05G002080)	*AfGpd*-for	CGAGAAGCCCGCCAAGT
		AfGpd-rev	AAAGGTGTCGGTCAAAGCAAT
AfLaeA	DFL_005107 (EVM02G003030)	RT-*AfLaeA*-for	TCTGGTAAACCACTTCGGTAC
		RT-*AfLaeA*-rev	GTGCGATGCAAAGGGAC
artA	DFL_002601 (EVM01G011780)	RT-*artA*-for	GCTCCCACATTTCCACCT
		RT-*artA*-rev	GGATTATCGGCATTCTGACTT
artB	DFL_002602 (EVM01G011790)	RT-*artB*-for	GCAGTTCAAACGCAGAGC
		RT-*artB*-rev	ATGGAAGAGCCGCAAAG
artC	DFL_002603 (EVM01G011800)	RT-*artC*-for	GTCCACGGAGGAAATAATCG
		RT-*artC*-rev	CTCGGAGAACTCGTACTTATCAA
artD	DFL_002604 (EVM01G011810)	RT-*artD*-for	TACTTGATGAGCCTCCACG
		RT-*artD*-rev	TTAGAAGACGCCCGATGA
artE	DFL_002606 (EVM01G011830)	RT-*artE*-for	TCACAAGATGGGCTCGGATTG
		RT-*artE*-rev	TAGCAGCGACGCCAGCAGT
artR	DFL_002600 (EVM01G011760)	RT-*artR*-for	CGAGACTTTGTGGGAGGA
		RT-*artR*-rev	ACTGTGGGACGTGATTGTG

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