

Supplementary Material

Title: Heteroexpression of *Aspergillus nidulans laeA* in marine-derived fungi triggers upregulation of secondary metabolite biosynthetic genes

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Az5LaeA 68 NGR1TYHGFR2RGMYFLPCDEQE3QDR4LDI5FHKL6FT7VAR8VSE9SLI10YAP11HP12GN13RF14LDL15GC16GT17
AnLaeA 77 NGR1TYHGFR2RGMYFLPCDEQE3QDR4LDI5FHKL6FT7VAR8VSE9SLI10YAP11HP12T13NG14RF15LDL16GC17GT18
PzLaeA 82 NGR1LYHGFR2RG3IY4PLPCDEQE5QDR6LDI7FHKL8FT9VAR10SDGLI11YAP12HP13S14NG15RV16LDL17GC18GT19
PoLaeA 63 NN1RL2YH3SY4RK5GT6YMLPCDDE7EQDR8LDI9FHKL10FT11VAR12SDGLI13YAP14HP15K16NS17R18LDL19GC20GT21
MrLaeA 83 NGR1IYHGFR2RG3IY4FLPCDDLE5QDR6LDI7FHK8VIT9VAR10SDALI11YSP12HPR13NG14RF15LDL16GC17GT18
BsLaeA 84 NGR1LYHGFR2RG3IYMLPCDEPE4QDR5LDI6FHKL7FEAR8VS9DGLI10YAP11HPEN12GR13FDL14GC15GT16
EgLaeA 116 NGR1QYHGFR2KG3VYMYPCDDQ4EMDR5LDI6FHKL7ITEAR8VS9DGLI10YAP11HPQ12NG13RV14LDL15GC16GT17
ReLaeA 90 NGR1IYHGFK2KG3VYFP4PCDEQE5QDR6LDI7FHK8VITEAR9IG10DRLI11YAP12HS13DDC14RV15LDL16GC17GT18

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PzLaeA 142 IWAIDVANKYPEAFVVGVDLAPIQPPNHPKNC1DFYAPFD2FESPW-AMGESWDLIHMQMG
PoLaeA 123 IWSIEVANKYPDSFVVGVDLAPIQPQNHPSNCD1FYAPFD2FESPW-ALGEDSWDLIHMQMG
MrLaeA 143 IWAIDVAQKYPDAFVVGVDLSPIQPLNS1PRNC2DFYAPFD3FESPW-ALGEDSWDLIHMQLG
BsLaeA 144 IWAIDIAHKYPNAHVVGVDLAPIQPSNRPKNC1DFYAP2WDFESH3W-SLGEDSWDVVHMQMG
EgLaeA 176 IWAIDVAKKYPDAFVLGVDLSPIQPPNY1PRNC2DFYAP3WYD4SPW5PSLGEDSWDV6IHLQMG
ReLaeA 150 IWAIDVANKYPKSEFVVGVDLSPIQPKNI1PKNC2DFYAP3WDFES4PW-NLGENY5WDLIHM6QMG

Az5LaeA 187 CGSVMGWPNLYRRIFSHLRPGAWFEQVEIDFEP1RCDDR--SLDGLALRH2WYQ3YLKQ4ATAE
AnLaeA 196 CGSVMGWPNLYRRIFAHLRPGAWFEQVEIDFEP1RCDDR--SLDGTALRH2WYD3CLKQ4ATAE
PzLaeA 201 SGSVASWPNLYRRIFAHLRPGAWFEQVEIDFEP1RCDDR--SLDGLALR2QWY3QCLKQ4ATEE
PoLaeA 182 SGSVASWPSLYRRIFSHLRPGAWFEQVEIDFEP1RCDDR--SLSNLALRH2WYTAL3KRATES
MrLaeA 202 CGSVVSWPSLYRRIFAHLRPGAWFEQVEIDFEP1RCDDR--SLEGLALH2HWY3QCLKQ4ATEE
BsLaeA 203 SGSVASWPSLYRRIFSHLRPGAWFEQVEIDFEP1RCDDR--TLEGSALR2SWY3WLKQ4ATED
EgLaeA 236 CGSVTSWPSLYRRVFAHLRPGAWFEQVEIDFEP1RCDDRS2LALDNTAL3R4RWY5TLLKQ6ATEQ
ReLaeA 209 AGSVTSWPSLYRRIFAHLRPGAWFEQVEIDFEP1RC2EGR--SLENTALS3RWY4YLRQ5ATEQ

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Figure S1. Multiple sequence alignment of Az5LaeA with its orthologs by ClustalX2.1. Abbreviations and accession numbers: Az5LaeA, *Aspergillus* sp. Z5 LaeA, BK011996; AnLaeA, *Aspergillus nidulans* LaeA, XP_658411; PoLaeA, *Penicillium oxalicum* LaeA, EPS25650; PzLaeA, *Penicillium zonata* LaeA, XP_022580130; MrLaeA, *Monascus ruber* LaeA, AY63188; BsLaeA, *Byssosclamyces spectabilis* LaeA, GAD98736; ReLaeA, *Rasamsonia emersonii* LaeA, XP_013330850; EgLaeA, *Elaphomyces granulatus* LaeA, OXV07344

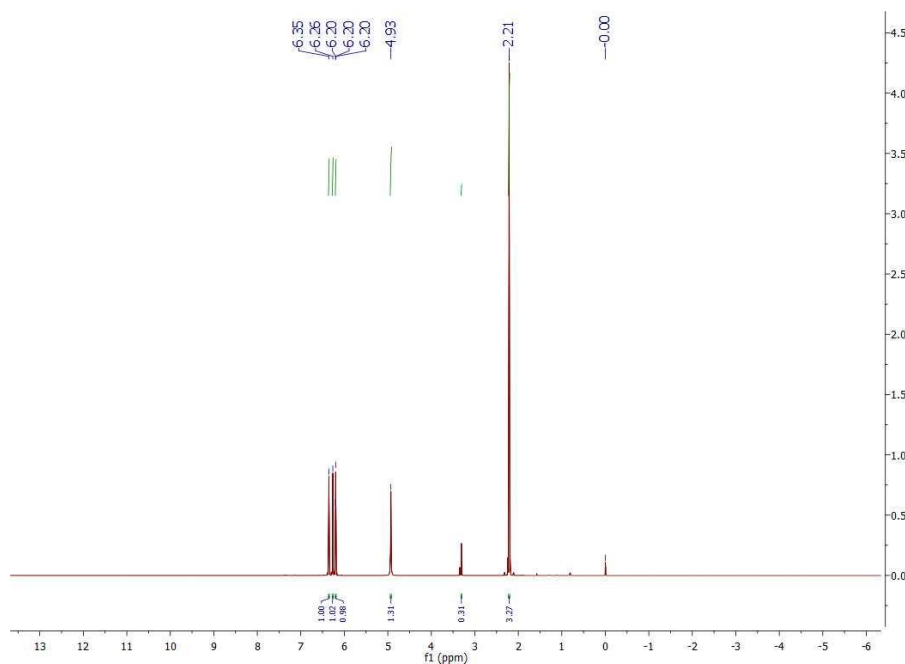


Figure S2. ¹H NMR Spectrum of compound 1 in CD₃OD

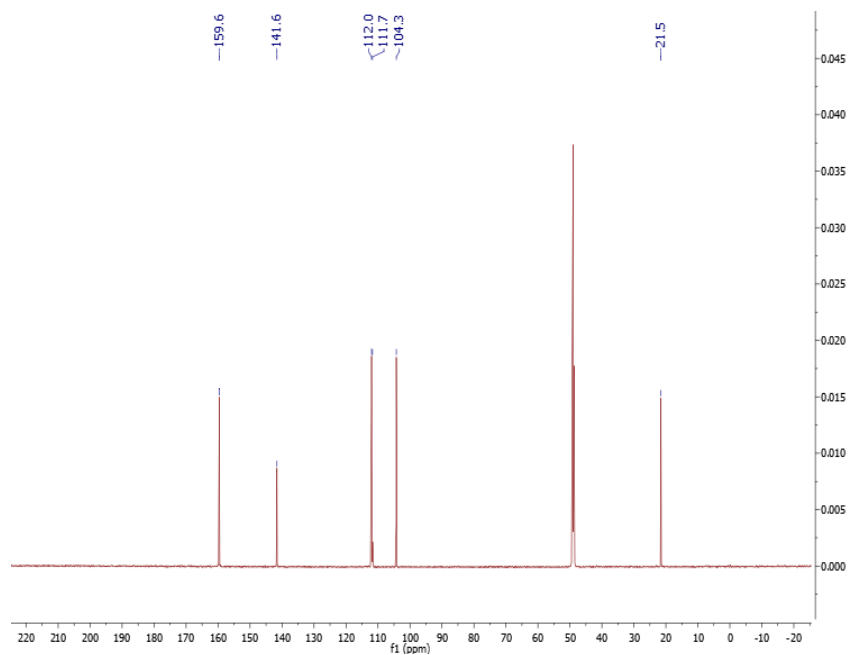


Figure S3. ^{13}C NMR Spectrum of compound 1 in CD_3OD

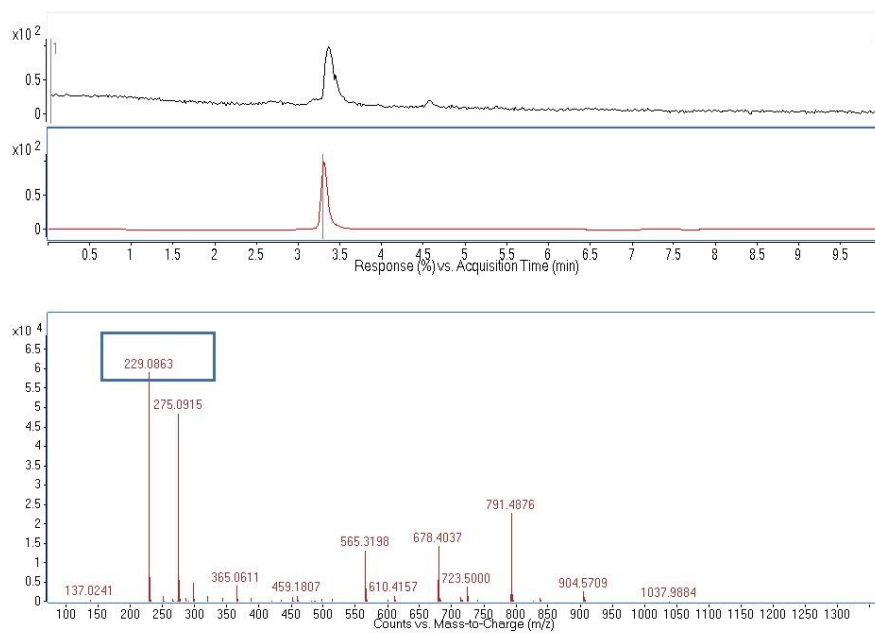


Figure S4. LC-MS analysis of compound 1

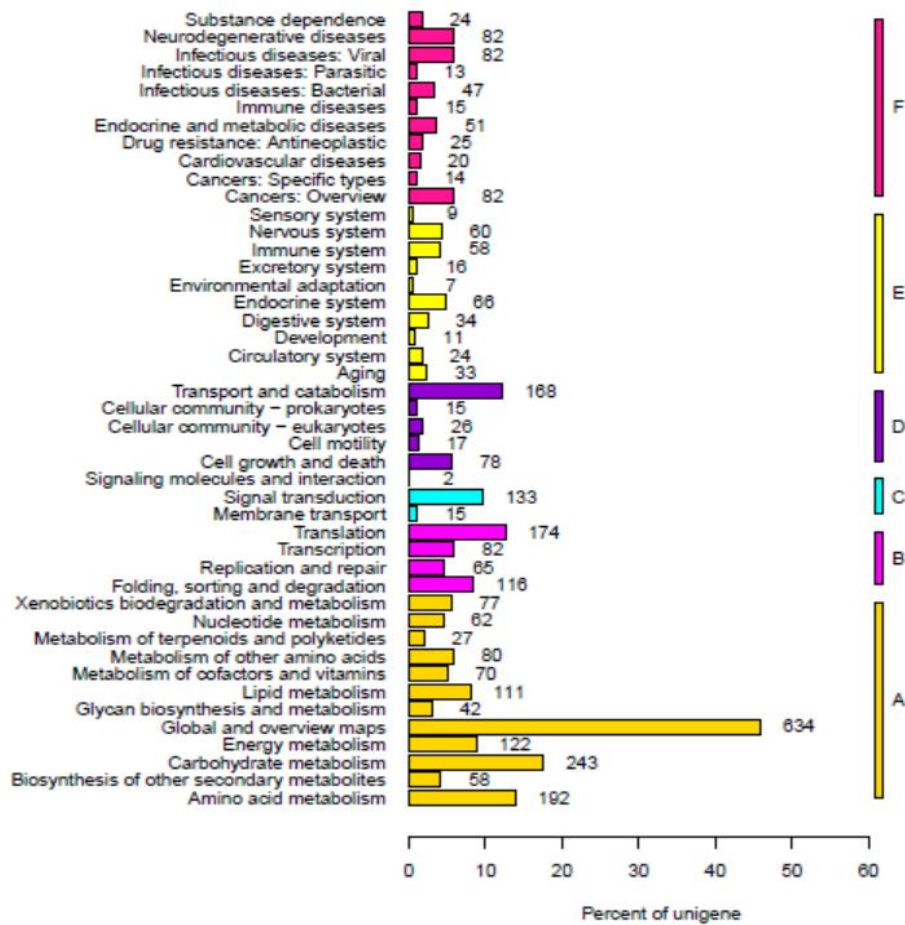


Figure S8. The KEGG pathway classification of assembled unigenes. (A) Metabolism; (B) Genetic Information Processing; (C) Environment Information Processing; (D) Cellular Process; (E) Organismal System; (F) Others

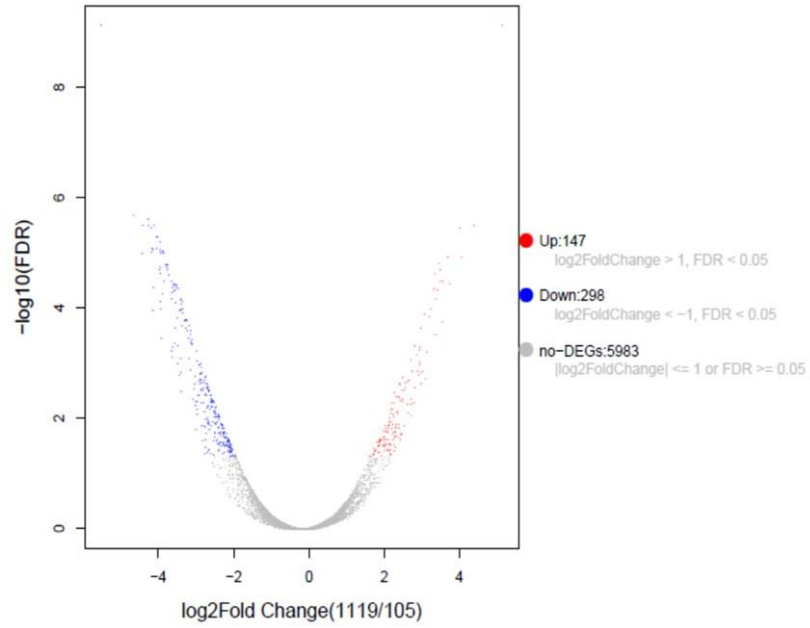


Figure S9. The visualization of differentially expressed genes (DEGs). Red dot: Significantly upregulated gene; Blue dot: Significantly down regulated gene; Grey dot: Not significantly differentially expressed gene.

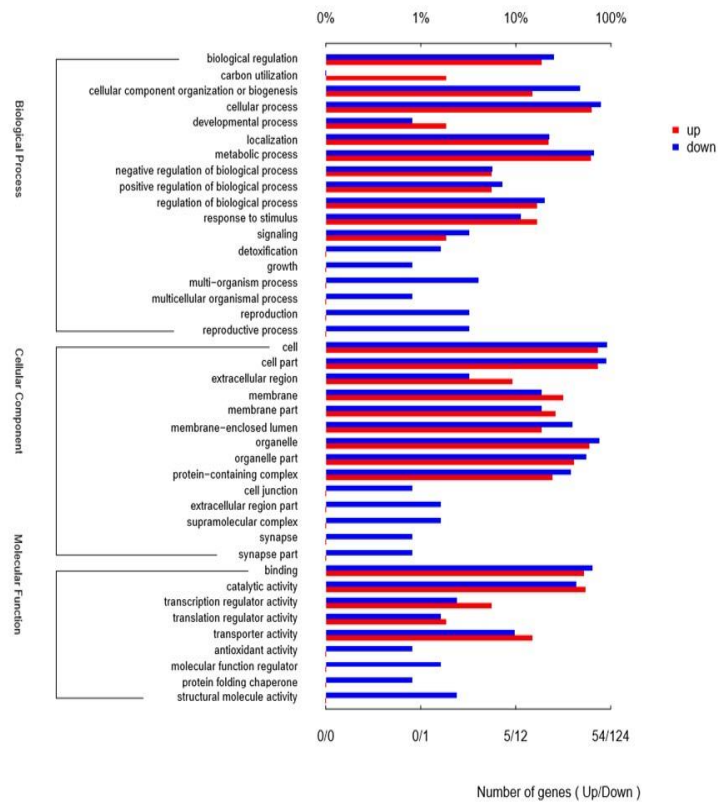


Figure S10. WEGO enrichment comparison between annotated unigenes and AnLaeA upregulated genes in transformant OE::AnLaeA^{11.19}.

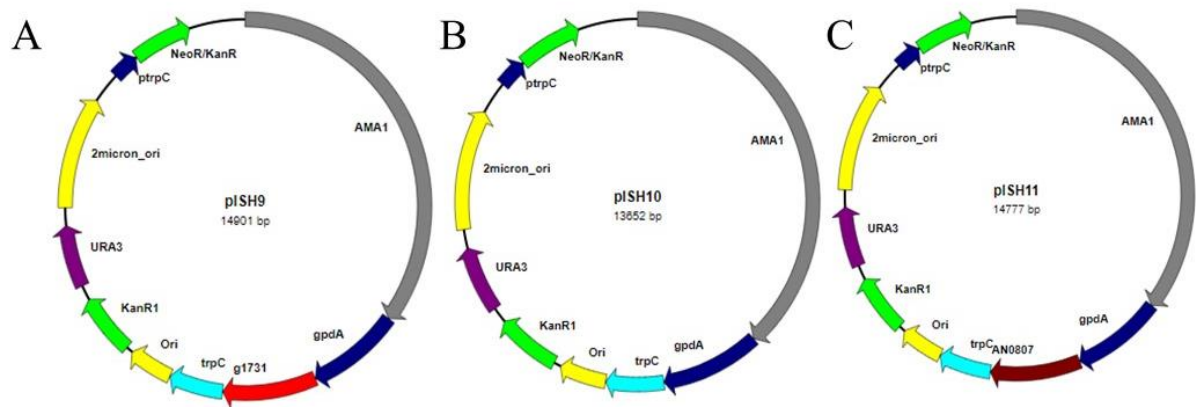


Figure S11. Plasmid maps. (A) *Aspergillus* sp. Z5 g1731.t1 *laeA* (Az5LaeA) overexpression plasmid; (B) Negative control plasmid; (C) *Aspergillus nidulans* AN0807 *laeA* (AnLaeA) overexpression plasmid

Table S1. Comparison between Az5LaeA and its orthologs

LaeA	Organism	Accession number	Length (aa)	Identity/coverage (%)
Az5LaeA	<i>Aspergillus</i> sp. Z5	BK011996	352	-
AnLaeA	<i>Aspergillus nidulans</i>	XP_658411	361	86.07/95
PoLaeA	<i>Penicillium oxalicum</i>	EPS25650	348	78.77/82
PzLaeA	<i>Penicillioopsis zonata</i>	XP_022580130	367	73.84/99
MrLaeA	<i>Monascus ruber</i>	AIY63188	368	71.10/93
BsLaeA	<i>Byssochlamys spectabilis</i>	GAD98736	369	70.75/96
ReLaeA	<i>Rasamsonia emersonii</i>	XP_013330850	377	66.67/89
EgLaeA	<i>Elaphomyces granulatus</i>	OXV07344	404	60.15/99

Table S2. Summary of RNA-seq statistics

	nc10.5	OE::AnLaeA ^{11.19}
Raw Reads		
Total Reads	50,033,398	48,146,046
Total Bases (bp)	7.51×10 ⁹	7.22×10 ⁹
Clean Reads		
Total Reads	49,979,906	48,075,956
Total Bases (bp)	7.33×10 ⁹	7.01×10 ⁹
Error %	0.0266	0.0263
Q20 %	97.13	97.18
Q30 %	93	93.26
GC %	52.83	52.31

Table S3. Statistics of the *de novo* assembly of RNA-seq

	Transcripts	Unigenes
Total number	33,747	18,923
Total Bases (bp)	71,916,686	25,898,836
GC %	51.22	51.24
Largest Length (bp)	26,466	26,466
Smallest Length (bp)	200	201
Average Length (bp)	2131.05	1368.64
N50 (bp)	4614	3348
N90 (bp)	1052	440

Table S4. Annotation of unigenes against different databases

Database / Type	Unigenes	Percentage %	Transcripts	Percentage %
Total number	18,923	-	33,747	-
NR	7662	40.49	16,866	49.98
Pfam	3792	20.03	9575	28.37
STRING	5313	28.08	11,985	35.51
SWISS-PROT	3910	20.66	8309	24.62
GO	3809	20.12	8113	24.04
KEGG	2117	11.19	5133	15.21

Table S5. Primers used in this study

Primers	Sequence *(5'-3')	Purpose
ISH-Y LaeA E1F1	ATGTTTGAGATGGGCCCGGTG	To clone coding sequence of
Y LaeA E2R2	TTATCTTAATGGTTTCCTAGCCTGG	AnLaeA gene
ISH Z5 LaeA F1	ATGTTTGAAAGGGTCCGGTG	To clone Az5LaeA gene
ISH Z5 LaeA R1	TTATCTCGACGGTTTTTCGCG	
ISH gpdA F1	GAAAAACTTCGTGCTATCACAAAACAGT ATACAAAAAATAGCCGCACCTTCAGTG GACT	To clone gpdA promoter with AMA1 flanking region.
ISH gpdA R2	TGTGATGTCTGCTCAAGCGGGGTAG	

Primers	Sequence *(5'-3')	Purpose
ISH gpdA R1	GGGGTCGAGGTCCCACCGGACCCTTTCC AAACATTGTGATGTCTGCTCAAGCGGG GTAG	To clone gpdA promoter with Az5LaeA gene flanking region
ISH gpdA R3	GGAGACGAGTTCCCACCGGGCCCATCTC AAACATTGTGATGTCTGCTCAAGCGGG GTAG	To clone gpdA promoter with AnLaeA gene flanking region
ISH TtrpC F1	AGCTTGACTAACAGCTACCCCGCTTGAG CAGACATCACAGGATCCACTTAACGTT ACTG	To clone TtrpC terminator with gpdA flanking region
ISH TtrpC F2	ATACTGCACATATAACCAGGCTAGGAAAC CATTAAAGATAAGGATCCACTTAACGTT ACTG	To clone TtrpC terminator with AnLaeA gene flanking region
ISH TtrpC F3	ATCCTACACATATAACCAAGCGCGAAAAC CGTCGAGATAAGGATCCACTTAACGTT ACTG	To clone TtrpC terminator with Az5LaeA gene and Ori flanking region
ISH TtrpC R1	ATTTTTGTGATGCTCGTCAGGGGGGCGG AGCCTATGGAAATAGAAAGAAGGATTA CCTC	
ISH-G418 F2 Y-G418 R1	TTCCATAGGCTCCGCCCCCTG CTTCGTGCTATCACAAAACAGTATACAA AAAATAAGCTTAAACGCTCTTTTCTCTT AGG	To clone vector backbone from pC-G418-YR vector with AMA1 flanking region
WJW-F WJW-R	GCAAGGTGTTTCAGAGACTA AGTGTGGAAGTGATAGCAT	To confirm AMA1 by diagnostic PCR
ISH Neo/Kan F1 ISH Neo/Kan R1	ATGATTGAACAAGATGGATTGC TCAGAAGAAGCTCGTCAAGAAG	To confirm G418 gene by diagnostic PCR
ISH gpdA_AnLaeA F1 ISH gpdA_AnLaeA R1	ACGCAGAGAGAAGGGCTGAGTAATA TGGTCGCCGGCTCTCGTTCA	To confirm gpdA-AnLaeA gene fragment by diagnostic PCR
ISH gpdA_Az5LaeA F1 ISH gpdA_Az5LaeA R1	GTAGGCAGCTTTGCCCGTCT TCGAAATCGAAGGGTGAATAGAAC	To confirm gpdA-Az5LaeA gene fragment by diagnostic PCR
Z5 VeA RT F1 Z5 VeA RT R1	CGAACGACCACTTTACAATGGCA GCCCGCTTATAGGCCATCT	To confirm Az5VeA gene expression by qPCR
Z5 VeIB RT F1	CCCTTCCAAGTGTTCTCTGCCA	

Primers	Sequence *(5'-3')	Purpose
Z5 VelB RT R1	CGTCCACGAGAGCCTTGAC	To confirm Az5VelB gene expression by qPCR
Z5afIR RTF1	AGTATCGCAGGGCAGGCTG	To confirm Az5afIR1 gene expression by qPCR
Z5afIR RTR1	GAGTCCCGAGATCCACAGCT	To confirm Az5afIR1 gene expression by qPCR
Z5afIR RTF2	AGAGGACGAGCGCAGGCTTA	To confirm Az5afIR2 gene expression by qPCR
Z5afIR RTR2	AACTGTTCCAGCGCCCCGT	To confirm Az5afIR2 gene expression by qPCR
Z5 sclR RTF1	CTCTACTTCACGCTCTTCGAAA	To confirm Az5sclR gene expression by qPCR
Z5 sclR RTR1	ACGTACGCCGCCATCAAAA	To confirm Az5sclR gene expression by qPCR
Z5 GcnE RTF1	TACAAGATCGACCCAGTAATGG	To confirm Az5GcnE gene expression by qPCR
Z5 GcnE RTR1	AACGGTGGCGACAGCTCGTT	To confirm Az5GcnE gene expression by qPCR
Z5 mtfA RTF1	AAGGCCTTCTCACGACCCT	To confirm Az5mtfA gene expression by qPCR
Z5 mtfA RTR1	TTCGTGGCGCTTCATATTGCT	To confirm Az5mtfA gene expression by qPCR
Z5 sltA RTF1	ATGGACTTCGCAGACTTCG	To confirm Az5sltA gene expression by qPCR
Z5 sltA RTR1	AGCAGAGTCATCTTCGAATTG	To confirm Az5sltA gene expression by qPCR
Z5 LlmF RTF1	ATCCAAAACACTGCACGAACTC	To confirm Az5LlmF gene expression by qPCR
Z5 LlmF RTR1	CACCTGGGCGCTTTCATACGA	To confirm Az5LlmF gene expression by qPCR
Z5 mcrA RTF1	AGGTCACACGCTTCTTTTACG	To confirm Az5mcrA gene expression by qPCR
Z5 mcrA RTR1	AAAAGATCCACGGGGCGAAG	To confirm Az5mcrA gene expression by qPCR
Z5 OrsA RTF1	CCGAGGATCTATCCGTAA	To confirm Az5OrsA gene expression by qPCR
Z5 OrsA RTR1	CCCAGACCACAGCGACAT	To confirm Az5OrsA gene expression by qPCR
Z5 OrsB RTF1	GAGCAAATCAGCGACAGC	To confirm Az5OrsB gene expression by qPCR
Z5 OrsB RTR1	TTAGGGCACCGACGAATC	To confirm Az5OrsB gene expression by qPCR
Z5 OrsC RTF1	ATTTGGACGGTTTGGCAGG	To confirm Az5OrsC gene expression by qPCR
Z5 OrsC RTR1	ATGTCCGGACTGAAATCAAACA	To confirm Az5OrsC gene expression by qPCR
Z5 OrsD RTF1	ACCCGGCAGTCTTGGTGGTG	To confirm Az5OrsD gene expression by qPCR
Z5 OrsD RTR1	CAGGAAGCCTCTCAGCAATAC	To confirm Az5OrsD gene expression by qPCR
Z5 OrsE RTF1	TTAATGACATTGCGACTCCC	To confirm Az5OrsE gene expression by qPCR
Z5 OrsE RTR1	AGCGCCTCATCGACTCTCA	To confirm Az5OrsE gene expression by qPCR
Z5 OrsF RTF1	AAACGTCAATGTAGGTTTCAGC	To confirm Az5OrsF gene expression by qPCR
Z5 OrsF RTR1	TCCCGATATCATCGCGCTCC	To confirm Az5OrsF gene expression by qPCR

*The italicized primer sequence is homologous 5' flanking region to adjacent gene in vector construction

Table S6. List of plasmids used in this study

Plasmid	Genotype	Reference
pISH1	<i>amp oriC AnLaeAE1E2</i>	This study
pISH9	<i>ama1 gpdA::Az5LaeA::trpC neoR/kanR</i>	This study
pISH10	<i>ama1 gpdA trpC neoR/kanR</i>	This study
pISH11	<i>ama1 gpdA::AnLaeA::trpC neoR/kanR</i>	This study
pC-G418	<i>Ori neoR/kanR URA3 2micron</i>	http://www.addgene.org/61767/
ANep2	<i>ama1 pyrG GlaPr lacA GlaTt pUC18</i>	http://fgsc.net/

Table S7. List of LaeA regulated transcription factors, diorcinol-synthesis and velvet complex genes in *Aspergillus* sp. Z5

Genes	<i>A. nidulans/</i> <i>A. oryzae</i>	<i>Aspergillus</i> sp. Z5 homologue (%Identity/%Positives/ Query coverage)	Function	Reference
Transcription factors				
<i>aflR</i>	AN7820	g378.t1 (29/42/94)	Sterigmatocystin biosynthesis regulator	[1]
<i>gcnE</i>	AN3621	g3373.t1 (94/96/99)	Acetyltransferase in SAGA complex	[2]
<i>llmF</i>	AN6749	g9402.t1 (76/83/96)	LaeA-like methyltransferase	[3]
<i>mcrA</i>	AN8694	g6964.t1 (68/74/98)	Master regulator of secondary metabolism in <i>Aspergillus</i>	[4]
<i>mtfA</i>	AN8741	g2250.t1 (78/85/99)	Putative C2H2 transcription factor involve in regulation of secondary metabolism	[5]
<i>sclR</i>	AO09001100 0215	g6252.t1 (65/70/25)	Hyphal morphology, asexual conidiospore formation and sclerotial production	[6]
<i>sltA</i>	AN2919	g7633.t1 (78/86/99)	C2H2 transcription factor involve in cation homeostasis	[7]

Genes	<i>A. nidulans/</i> <i>A. oryzae</i>	<i>Aspergillus</i> homologue (%Identity/%Positives/ Query coverage)	sp. Z5	Function	Reference
Diorcinol biosynthesis					
<i>orsA</i>	AN7909	g2173.t1 (77/85/100)		Polyketide synthase; secondary metabolite gene cluster	F9775 [8]
<i>orsB</i>	AN7911	g2171.t1 (85/92/98)		Putative amidohydrolase; secondary metabolite gene cluster	F9775 [8]
<i>orsC</i>	AN7912	g2170.t1 (79/87/98)		Putative tyrosinase; secondary metabolite gene cluster	F9775 [8]
<i>orsD</i>	AN7913	g2169.t1 (64/75/98)		F9775 secondary metabolite gene cluster	[9]
<i>orsE</i>	AN7914	g2168.t1 (87/93/99)		Putative alcohol dehydrogenase; secondary metabolite gene cluster	F9775 [9]
<i>orsF</i>	AN7910	g2172.t1 (78/88/93)		Hypothetical protein	[10]
Velvet complex					
<i>veA</i>	AN1052	g4963.t1 (73/79/99)		Coordinates development and secondary metabolism in response to light	[11]
<i>velB</i>	AN0363	g142.t1 (80/87/95)		Coordinates development and secondary metabolism in response to light	[11]

References

1. Bok, J.W.; Keller, N.P. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot. cell* **2004**, *3*, 527-535, doi:10.1128/ec.3.2.527-535.2004.

2. Georgakopoulos, P.; Lockington, R.A.; Kelly, J.M. The Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex in *Aspergillus nidulans*. *PloS one* **2013**, *8*, e65221-e65221, doi:10.1371/journal.pone.0065221.
3. Palmer, J.M.; Theisen, J.M.; Duran, R.M.; Grayburn, W.S.; Calvo, A.M.; Keller, N.P. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. *PLoS Genet.* **2013**, *9*, e1003193, doi:10.1371/journal.pgen.1003193.
4. Oakley, C.E.; Ahuja, M.; Sun, W.-W.; Entwistle, R.; Akashi, T.; Yaegashi, J.; Guo, C.-J.; Cerqueira, G.C.; Russo Wortman, J.; Wang, C.C.C., et al. Discovery of McrA, a master regulator of *Aspergillus* secondary metabolism. *Mol. Microbiol.* **2017**, *103*, 347-365, doi:10.1111/mmi.13562.
5. Ramamoorthy, V.; Dhingra, S.; Kincaid, A.; Shantappa, S.; Feng, X.; Calvo, A.M. The putative C2H2 transcription factor MtfA is a novel regulator of secondary metabolism and morphogenesis in *Aspergillus nidulans*. *PloS one* **2013**, *8*, e74122-e74122, doi:10.1371/journal.pone.0074122.
6. Jin, F.J.; Takahashi, T.; Matsushima, K.-i.; Hara, S.; Shinohara, Y.; Maruyama, J.-i.; Kitamoto, K.; Koyama, Y. SclR, a basic helix-loop-helix transcription factor, regulates hyphal morphology and promotes sclerotial formation in *Aspergillus oryzae*. *Eukaryot. cell* **2011**, *10*, 945-955, doi:10.1128/EC.00013-11.
7. Shantappa, S.; Dhingra, S.; Hernández-Ortiz, P.; Espeso, E.A.; Calvo, A.M. Role of the zinc finger transcription factor SltA in morphogenesis and sterigmatocystin biosynthesis in the fungus *Aspergillus nidulans*. *PloS one* **2013**, *8*, e68492-e68492, doi:10.1371/journal.pone.0068492.
8. Sanchez, J.F.; Chiang, Y.-M.; Szewczyk, E.; Davidson, A.D.; Ahuja, M.; Elizabeth Oakley, C.; Woo Bok, J.; Keller, N.; Oakley, B.R.; Wang, C.C.C. Molecular genetic analysis of the orsellinic acid/F9775 gene cluster of *Aspergillus nidulans*. *Mol. Biosyst.* **2010**, *6*, 587-593, doi:10.1039/b904541d.
9. Bok, J.W.; Chiang, Y.-M.; Szewczyk, E.; Reyes-Dominguez, Y.; Davidson, A.D.; Sanchez, J.F.; Lo, H.-C.; Watanabe, K.; Strauss, J.; Oakley, B.R., et al. Chromatin-level regulation of biosynthetic gene clusters. *Nat. Chem. Biol.* **2009**, *5*, 462-464, doi:10.1038/nchembio.177.
10. Galagan, J.E.; Calvo, S.E.; Cuomo, C.; Ma, L.J.; Wortman, J.R.; Batzoglou, S.; Lee, S.I.; Baştürkmen, M.; Spevak, C.C.; Clutterbuck, J., et al. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* **2005**, *438*, 1105-1115, doi:10.1038/nature04341.
11. Bayram, O.; Krappmann, S.; Ni, M.; Bok, J.W.; Helmstaedt, K.; Valerius, O.; Braus-Stromeier, S.; Kwon, N.J.; Keller, N.P.; Yu, J.H., et al. VelB/VeA/LaeA complex coordinates light signal

with fungal development and secondary metabolism. *Science* **2008**, *320*, 1504-1506,
doi:10.1126/science.1155888.