

Supplementary materials

Deletion of a Rare Fungal PKS CgPKS11 Promotes Chaetoglobosin A Biosynthesis, Yet Defers the Growth and Development of *Chaetomium globosum*

Biyun Xiang ¹, Xiaoran Hao ^{2,*}, Qiaohong Xie ^{1,3}, Guangya Shen ¹, Yanjie Liu ¹ and Xudong Zhu ^{1,*}

¹ Beijing Key Laboratory of Genetic Engineering Drug and Biotechnology, College of Life Sciences, Beijing Normal University, Beijing 100875, China

² National Experimental Teaching Demonstrating Center, College of Life Sciences, Beijing Normal University, Beijing 100875, China

³ Xiamen No.1 High School of Fujian, Xiamen 361000, China

* Correspondence: zhu11187@bnu.edu.cn (X.D.Z); 2015xrhao@bnu.edu.cn (X.R.H); Tel: +86-010-58804266 (X.D.Z); Tel: +86-010-58802159-812

ASSOCIATED CONTENT

Supplementary Tables and Figures

Table S1. Putative PKSs presented in genomes of *C. globosum*.

Table S2. Primers used in this study.

Table S3. Expression of a predicted standalone ACP encoding gene CHGG_09364.

Figure S1. Construction of *Cgpks11* deletion plasmid.

Figure S2. Diagnostic PCR screening for *Cgpks11* deletion mutants.

Figure S3. Southern blotting to confirm that Cas9 was eliminated in $\Delta cgpks11$ mutants.

Figure S4. Effect of *Cgpks11* deletion on pigment biosynthesis during the liquid fermentation.

Table S1. Putative PKSs presented in genomes of *C. globosum*.

Gene in NK102	Gene in CBS148.51	Type of PKS	Location
<i>Cgpks1</i>	CHGG_00542	Type I PKS	Scaffold_1
<i>Cgpks2</i>	CHGG_03101	Type I PKS	Scaffold_2
<i>Cgpks3</i>	CHGG_05147	Type I PKS	Scaffold_4
<i>Cgpks4</i>	CHGG_09585	Type I PKS	Scaffold_7
<i>Cgpks5</i>	CHGG_04475	Type I PKS	Scaffold_4
<i>Cgpks6</i>	CHGG_08746	Type I PKS	Scaffold_6
<i>Cgpks7</i>	CHGG_05358	NRPS-Type I PKS	Scaffold_3
<i>Cgpks8</i>	CHGG_09586	Type I PKS	Scaffold_7
<i>Cgpks9</i>	CHGG_10092	Type I PKS	Scaffold_7
<i>Cgpks10</i>	CHGG_00046	Type I PKS	Scaffold_1
<i>Cgpks11</i>	CHGG_10647	Type I PKS	Scaffold_7
<i>Cgpks12</i>	CHGG_03589	Type I PKS	Scaffold_2
<i>Cgpks13</i>	CHGG_02374	Type I PKS	Scaffold_2
<i>Cgpks14</i>	CHGG_01021	Type I PKS	Scaffold_1

<i>Cgpks15</i>	CHGG_04068	Type I PKS	Scaffold_4
<i>Cgpks16</i>	CHGG_05286	NRPS-Type I PKS	Scaffold_3
<i>Cgpks17</i>	CHGG_05113	Type I PKS	Scaffold_4
<i>Cgpks18</i>	CHGG_10027	Type I PKS	Scaffold_7
<i>Cgpks19</i>	CHGG_10128	Type I PKS	Scaffold_7
<i>Cgpks20</i>	CHGG_07645	Type I PKS	Scaffold_5
<i>Cgpks21</i>	CHGG_01239	NRPS-Type I PKS	Scaffold_1
<i>Cgpks22</i>	CHGG_07638	Type I PKS	Scaffold_5
<i>Cgpks23</i>	CHGG_00087	Type I PKS	Scaffold_1
<i>Cgpks24</i>	CHGG_08147	Type I PKS	Scaffold_5
<i>Cgpks25</i>	CHGG_08793	Type I PKS	Scaffold_6
<i>Cgpks26</i>	CHGG_08141	Type I PKS	Scaffold_5
<i>Cgpks27</i>	CHGG_00246	Type I PKS	Scaffold_1
<i>Cgpks28</i>	CHGG_08934	Type III PKS	Scaffold_6

Table S2. Primers used in this study.

Primer name	Sequence (5'-3')
act1p-F	GCAGGCATGCAAGCTCATCGTATGGTCGTTGAAACC
act1p-R	CTTCTTGCCCCCATTGACGGCTGGAAAGGTGC
cas9in-F	ATGGCCCCAAAGAAGAACG
cas9in-R	GGCCAGTGCCAAGCTTCCCCAGCATGCCTGCTATT
CXcas9infu-1	ATCCGGTTCTCCGTCTGGT
u6gdna1-F	CGAAATTGAGCTCGCGATGCAATGCAGCTGGAA
u6gdna1-R	CGGTCTTCATGAAGACTAGAGGAAAGAAAGAGA
u6gdna2-F	TAGTCTTCATGAAGACC GGTTAGAGCTAGAAATAGCAA
u6gdna2-R	GTT
Hyg-Xba I-F	CTAGAGGATCCCCGG<u>CTCGAG</u>TAAAACAAAAAGCAC
Hyg-Xho I-F	TTTTGTTTACT<u>CGAGT</u><u>CTAG</u>AGTCGACAGAAGATGA
Hyg-Xho I-R	GGATCCCCGG<u>CTCGAG</u>AAAGAAGGATTACCTCTAAACAAG
PCgpks11-UF	TTTACTCGAGTCTAGCTACGAAACAAGCAGGATGGATGG
PCgpks11-UR	TTCTGTCGACTCTAGGCCCTACGGATACAATCTTCTTGG
PCgpks11-DF	CCGGGGATCCTCTAGGACACCGTTTCTTGAGTGCC
PCgpks11-DR	TAGAGGATCCT<u>CTAG</u>AGCGTGTAAAGCAATATCAGCG
PCgpks11-N19-F	CCTCGATGCCGTAACCTCCCC
PCgpks11-N19-R	AAACAAGGGGAGGTACCGGCATC
P11-KO-VP-F	ATAGGTTGTAGGTGGGCTG
P11-KO-VP-R	CGTTCTGGCTCATACTCAC
iHYG-R	GCAAAGTGCCGATAAACAT
PHYG-ter-F	TGAATGCTCCGTAACACC
HYG-F	CTAGAGTCGACAGAAGATGA
HYG-R	AAAGAAGGATTACCTCTAAACAAGT
qCgpks11-F	CGCTATCTACCTCAAGAAGTCA
qCgpks11-R	GAGGTTACCGGCATTCTGT
q10646-F	CATTCTCTGGCTGACCTTG
q10646-R	GACTTCATCTCTCATCTCTGT
q10649-F	TCAAGCACCAATCTCCAGTT
q10649-R	GGCTTCTCAAAGTCGGTTC
q10650-F	GATACTTGTACCGAGGTC
q10650-R	CGAGGCCTAGAACATATC
PCas9-PF	CCCACCATCTACCACCTGA

PCas9-PR	GCCGCCAGTAGTTCTTCAT
qGAPDH-F	AACGGCAAGAAGGTCAAG
qGAPDH-R	TCTCGGTGGTAGTGAACA
q01237-F	GGGAAGGACCGATACCATAAAC
q01237-R	TCTAAACCCCATTCTACAACCG
q01238-F	CAGAGGGATGTGGTAAGGG
q01238-R	CTAACGTATATTATAAGCGAGCGA
q01239-F	GATTTCGCCTCGGTTGTGCTTA
q01239-R	CATAGTGATACCTTGCCTCTCC
q01240-F	GGTATTACAACGGATGCGACTT
q01240-R	CGGTAGGAGAACACGCTGAC
q01241-F	GAGGAATGGCACCAAGGAAT
q01241-R	AGTTAACGGCAGCATCT
q01242-1-F	GGCTCCAAGCATAACGCAG
q01242-1-R	TGGTGTTGTTGGGTCTCC
q01242-2-F	GCCGTGTCCGAGAGTTATGT
q01242-2-R	AGAGGAAAGCCAGCAGTTG
q01243-F	CGGTCTTGCAGCTATTGAT
q01243-R	GCTGGCGACTTCTTGTCTG
q01244-F	CACACGCAACGAGTATATCCT
q01244-R	ATCGTGCTTGCCGCTTC
q00542-F	ATCTTCCGCCTAACCGA [19]
q00542-R	GTCCTTCGTTCTGGGTTGTC [19]

* The bold parts indicate the homologous arms of the primers. The underlined part represents the reserved restriction enzyme site.

Table S3. Expression of a predicted standalone ACP encoding gene CHGG_09364.

Gene symbol	Deduced function	FPKM_WT1	FPKM_WT2	FPKM_WT3	pval
CHGG_09364	ACP domain	1.53394	1.45815	1.37813	0.004947391

*FPKM: Fragments per Kilobase Million; WT1/WT2/WT3: Three culture replicates of wild-type strains have been used.
pval: *p*-value.

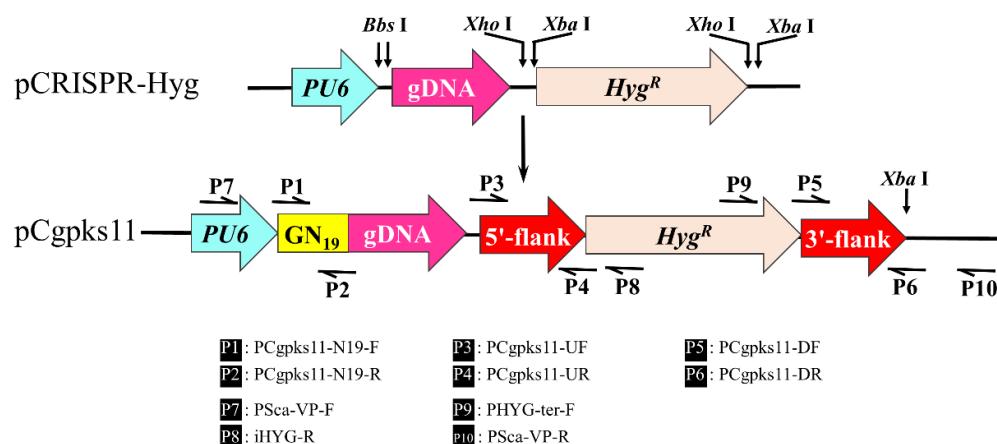


Figure S1. Construction of *Cgpk11* deletion plasmid. Primers for plasmid construction or diagnostic PCR are indicated in black boxes.

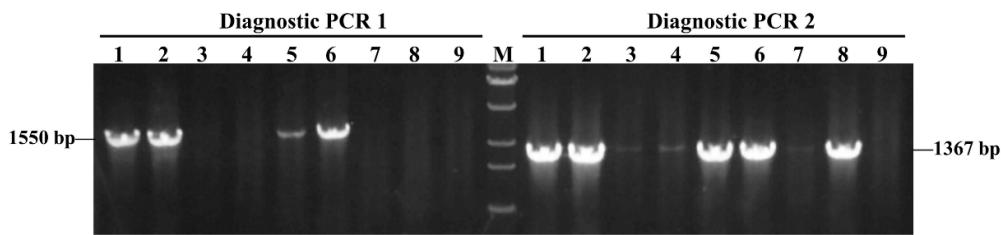


Figure S2. Diagnostic PCR screening for *Cgpks11* deletion mutants. A 1550 bp fragment could be amplified by primers P11-KO-VP-F/iHYG-R, and a 1367 bp fragment could be amplified by primers PHYG-ter-F/P11-KO-VP-R. Lanes 1–9 were loaded samples from transformants NO11–19. M: Trans15K DNA Marker.

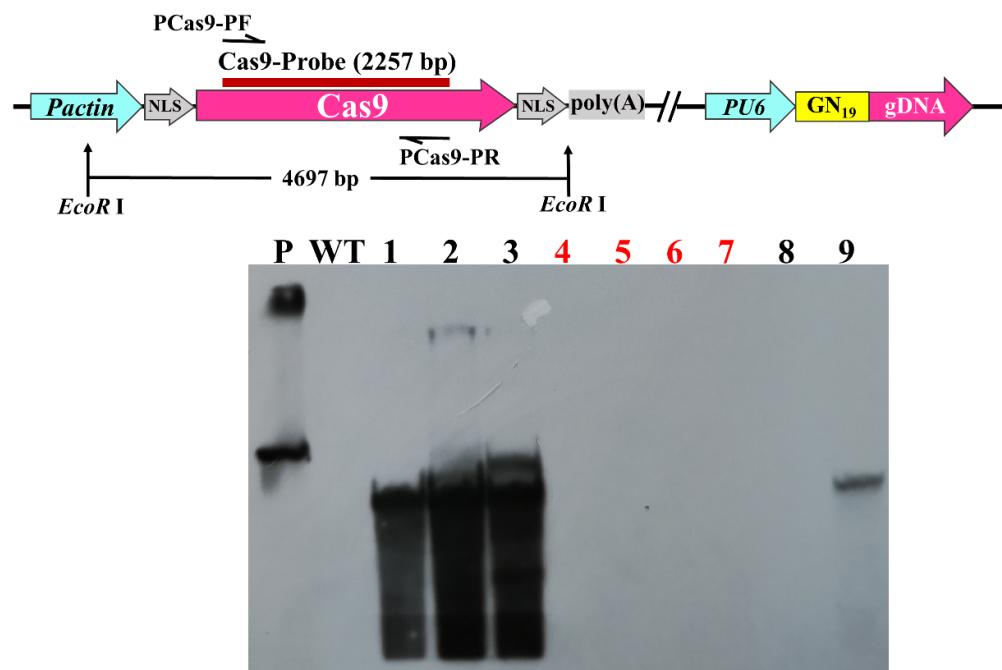


Figure S3. Southern blotting to confirm that Cas9 was eliminated in $\Delta cgpk11$ mutants. Cas9-probe was labeled and used as the probe, the unlinearized Cas9-containing plasmid pCRISPR-Hyg was used as the control. Lanes 1–9 were loaded samples from randomly selected transformants NO1, NO3, NO9, NO11, NO12, NO15, NO16, NO23 and NO33.

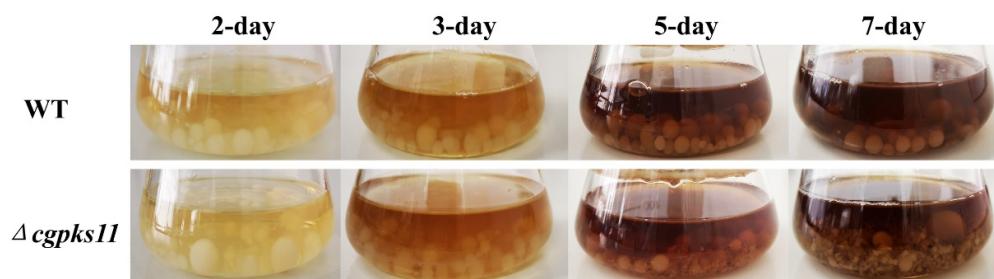


Figure S4. Effect of *Cgpks11* deletion on pigment biosynthesis during the liquid fermentation.