Supporting Information File S1

Cla4A, a Novel Regulator of Gene Expression Networks Required for Asexual and Insect-Pathogenic Lifecycles of *Beauveria bassiana*

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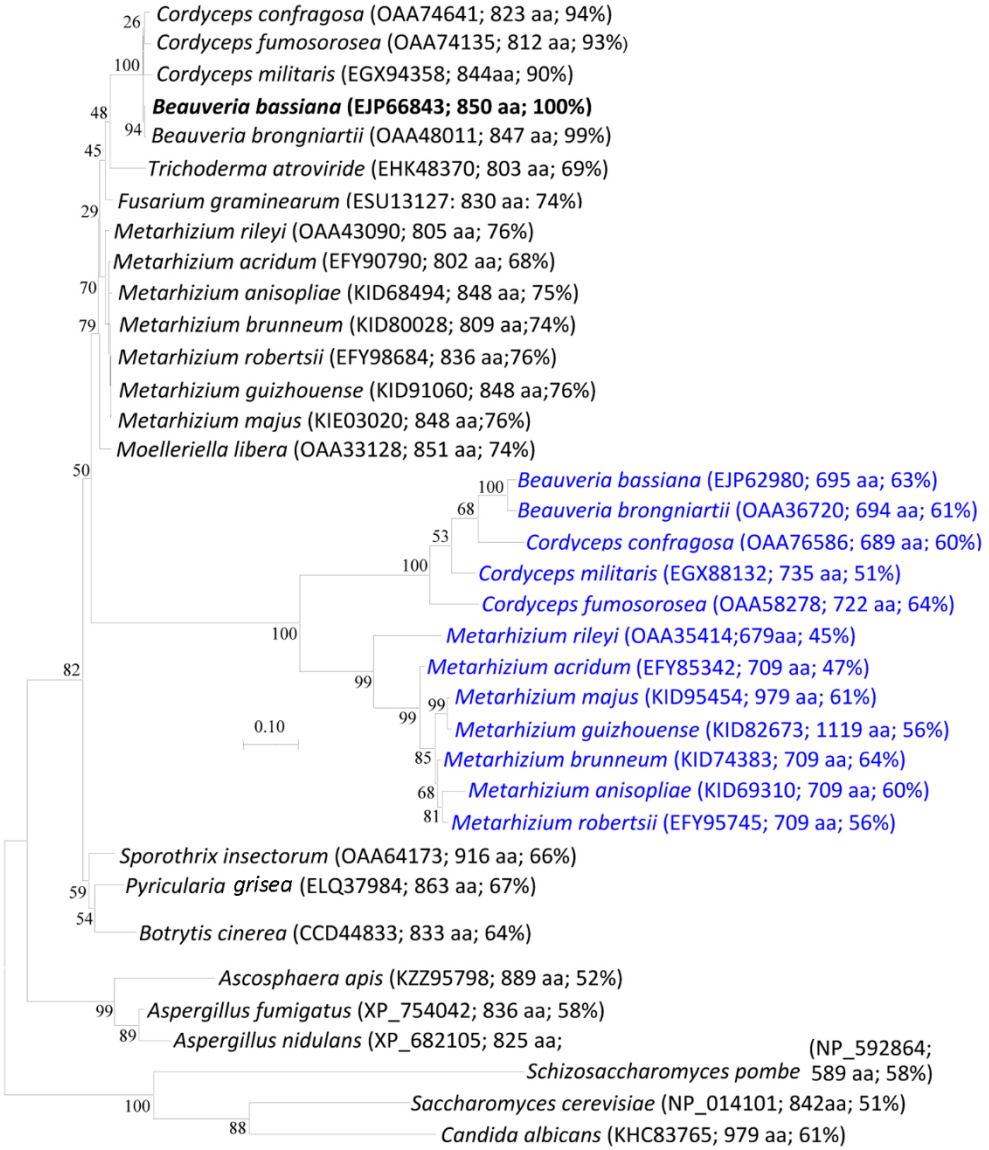


Figure S1. Phylogenetic tree of Cla4 homologs found in representative lineages of entomopathogenic and non-entomopathogenic ascomycetes. The tree was constructed with the maximum likelihood method in MEGA11 (http://www.megasoftware.net/). Bootstrap values of 1000 replications are shown at nodes. Scale bar: branch length proportional to genetic distance. The NCBI accession code and anomic acid sequence length of each homolog and its sequence identity to *B. bassiana* Cla4A (in bold) are given in the parentheses following the name of each fungus. Note that Cla4A and Cla4B (middle cluster in blue) exist only in the *Beauveria*/*Cordyceps* and *Metarhizium* lineages.

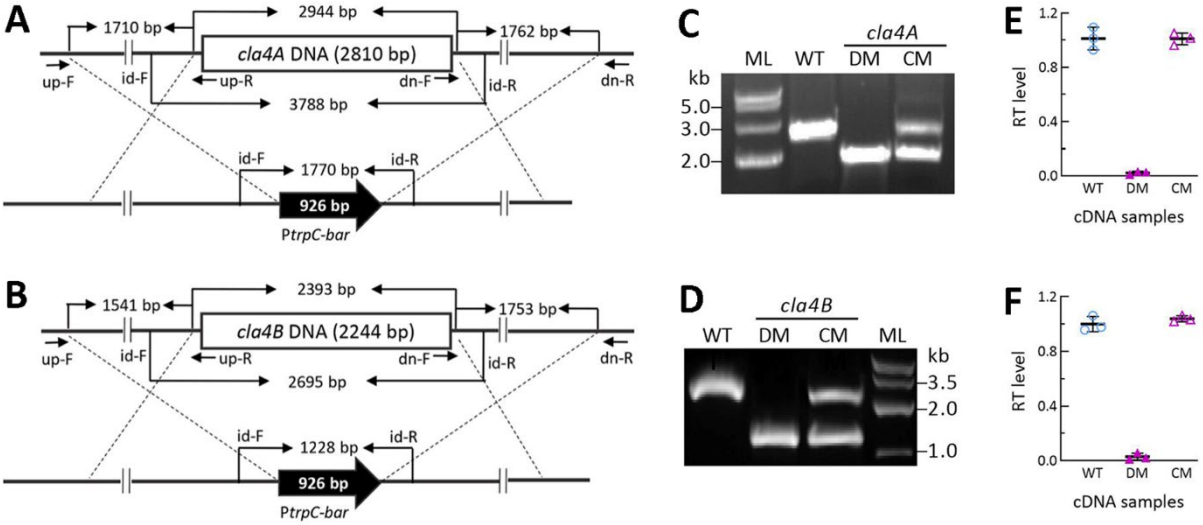


Figure S2. Generation and identification of *cla4A* and *cla4B* mutants in *B. bassiana*. (**A, B**) Schematic diagrams for deletion strategies of *cla4A* and *cla4B*. (**C, D**) The *cla4A* and *cla4B* mutants identified via PCR analysis. The DNA fragments detected in wild-type (WT), deletion mutant (DM) and complementation mutant (CM) indicate that a DNA fragment comprising partial flanking and full-length coding regions of each target gene was deleted in the DM strain as expected (3788 + 926 – 1770 = 2944 bp for *cla4A* deletion; 2695 + 926 – 1228 = 2393 bp for *cla4B* deletion). ML, molecular ladder of genomic DNA. (**E, F**) Relative transcript (RT) levels of *cla4A* and *cla4B* in the cDNA samples of their mutants with respect to the WT standard. Note that either *cla4A* or *cla4B* expression was abolished in the DM strain but restored to the WT level in the CM strain. Error bars: standard deviations of the means from three cDNA samples derived from independent 3-day-old SDAY cultures of each strain grown at an optimal regime.

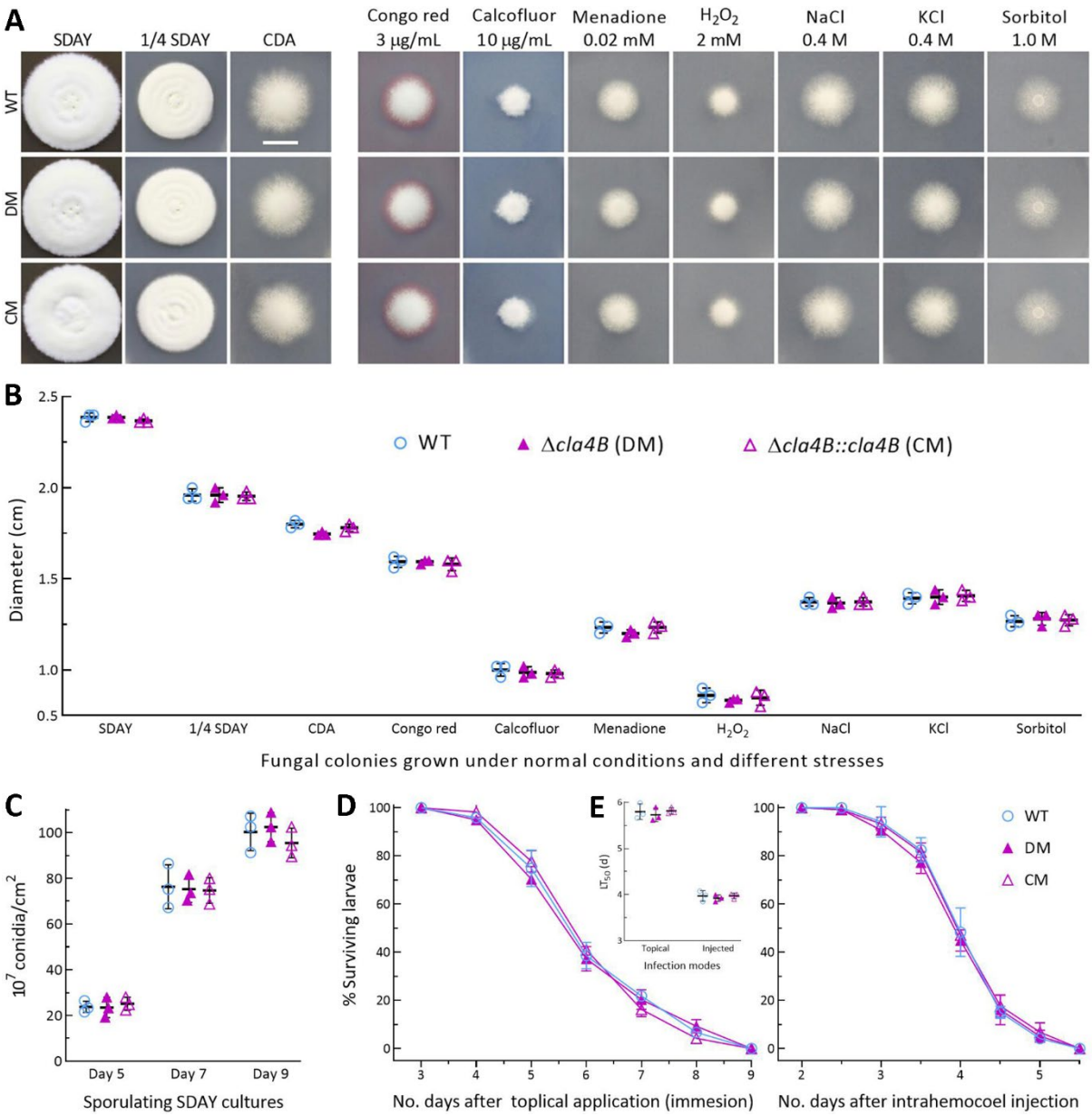


Figure S3. Dispensable role of Cla4B in the lifecycles *in vitro* and *in vivo* of *B. bassiana*. (**A, B**) Images and diameters of fungal colonies incubated 7 days at the optimal regime of 25°C and 12:12 (L:D) after initiation of colony growth by spotting 1 μL aliquots of a 10^6 conidia/mL suspension on the plates of rich media (SDAY and 1/4 SDAY) and the minimal medium CDA alone or supplemented with indicated concentrations of chemical stressors. (**C**) Conidial yields assessed from the -5, 7- and 9-day-old SDAY cultures of tested strains, which were initiated by spreading 1 μL of a 10^7 conidia/mL suspension per plate at the optimal regime. (**D, E**) Percent survival trends of *Galleria mellonella* larvae over the days after inoculation by topical application (immersion) of a 10^7 conidia/mL suspension or intrahemocoel injection of ~500 conidia per larva and median lethal time (LT_{50}) estimates made by modeling analysis of the trends. Note that none of examined phenotypes differed significantly among the tested strains. Error bars: standard deviations of the means from three independent replicates.

Table S1. Paired primers used for manipulation and detection of targeted genes in *B. bassiana*.

Primers	Paired sequences (5′–3′)*	Purpose
Cla4A-F/R	ACGAGCTGTACAAGTAA**CCCGGG**ATGTCACAGAACAGCGTATACTC / TGGCTGCAG GTCGAC**GGATCC**TTATTTGGCATGCTTCTTGAA	Cloning *cla4A* cDNA (2553 bp) for fusion to *GFP*
upCla4A-F/R	ACGAGCTGTACAAGTAA**CCCGGG**AGGGCATTGATGGTTCGT / TGGCTGCAGGTCG AC**GGATCC**AATCGGAAAGGCTCGTCA	Cloning *cla4A* 5′ fragment (1710 bp) for recombination/deletion
dnCla4A-F/R	GACCCATGGCTCGAG**TCTAGA**AAGGGCGGATACACGAC / GGTGGTGGTGGCTAGC **GTTAAC**AGAAAGGACGAGGCTGTG	Cloning *cla4A* 3′ fragment (1762 bp) for recombination/deletion
flCla4A-F/R	ATCCGTCGACCTGCAGCC**AAGCTT**AGGGCATTGATGGTTCGT / ACACTAGTCAGAT CTTC**TAGTGT**CGGATGACGCAACAACG	Cloning full-length *cla4A* sequence for complementation (4990 bp)
upCla4B-F/R	ACGAGCTGTACAAGTAA**CCCGGG**AACGCCTTGGTGTTTGCC / TGGCTGCAGGTCG AC**GGATCC**TTGAAGCCGCCTTTGAGC	Cloning *cla4B* 5′ fragment (1541 bp) for recombination/deletion
dnCla4B-F/R	GACCCATGGCTCGAG**TCTAGA**TTGCGTAGGCTGTTCTCGG / GGTGGTGGTGGCTA GC**GTTAAC**TCGCCGTCTTCAAATCCAC	Cloning *cla4B* 3′ fragment (1753 bp) for recombination/deletion
flCla4B-F/R	ATCCGTCGACCTGCAGCC**AAGCTT**AGGTATTCTGGCGGGTCC / ACACTAGTCAGAT CTTC**TAGTGT**CAAATGGGTCCGTAAAGAAAA	Cloning full-length *cla4B* sequence for complementation (4786 bp)
pCla4A-F/R	TCTATTTCCTCCATCTCCTTCC / ATCGTTCAATCGTCGCAAT	PCR detecting *cla4A*
pCla4B-F/R	GCAAATAAAACCTGCTAATGTCG / CCAAGGTCTACTCGGAAAACAAA	PCR detecting *cla4B*
qCla4A-F/R	CCCACCGACAGACATAGGCT / GCCCAGCCTTCCTTCTTGAT	qPCR detecting *cla4A*
qCla4B-F/R	AGAAGTCGCGTCCCTGAAAG / CAACTTCTTTGGTGCCGGTG	qPCR detecting *cla4B*
qBrlA-F/R	CGGCCGTTACTACATCCAGG / ACTCCGTTTCCATCGCACTT	qPCR detecting *brlA*
qAbaA-F/R	CCACGGCATGAACCTGTTTG / CGTTCGAGGGCAAAAAGTGG	qPCR detecting *abaA*
qWetA-F/R	AAGCCGGTGGGTTTACACTT / TTTGTCTTGGAGCTTCCGCT	qPCR detecting *wetA*
qActin-F/R	GGCAACATTGTCATGTCTGG / TTTGCTGGAAGGTGGATAGG	qPCR detecting β-actin gene
Pcdc24-F/R	CGCAACGCAGTCTTTTACA / CTTGGTCAAGCCTCCGAC	Cloning *cdc24* promoter (1590 bp)
Pcdc42-F/R	ACCGAGGGAGGAGCTGTTC / CGGCCATGGTTCAGATCTG	Cloning *cdc42* promoter (1627 bp)
Pste11-F/R	AAATAGGTGATGTCTGATAATGGC / TGGTGGCGGTTGCGTCT	Cloning *ste11* promoter (1633 bp)
Pste20-F/R	TGCTGGTAAATAGTGATGAACAAGTT / TTGTCCATGTTGGGGGGG	Cloning *ste20* promoter (1610 bp)
Pbck1-F/R	TACGATATGGACGATAAGAAGGC / CATGTTGGGCGACGTTTG	Cloning *bck1* promoter (1698 bp)
Psmp1-F/R	TGTTGGTGAATGAGTCGGATA / ACCCATGGTGACTGGATTGTA	Cloning *smp1* promoter (1578 bp)
PsskB-F/R	AAGCATCGCTACCTCGTGG / GCACGGTTGTCCATTGTCTT	Cloning *sskB* promoter (1644 bp)
Ppbs2-F/R	CTGGCATTATCCCGACTGTG / CATCGAGGTTTCGAGTTCTTTT	Cloning *pbs2* promoter (1678 bp)
Pmsn2-F/R	GACGGCTTCCTACTCCAACC / TCCATTGTGAGTGATTGTTTCG	Cloning *msn2* promoter (1584 bp)
Pfpr3-F/R	GAGCACAGCCACTTCAGACC / CAGCCATTTTGAAGATGGAGAT	Cloning *fpr3* promoter (1810 bp)
Pcrz1-F/R	CCTAACCCTACTGATAACTCCGA / GTCGAGAAGATGAGAGAAAGCG	Cloning *crz1* promoter (1629 bp)
Pvcx3-F/R	GGCGGGCTATCTGGAGTAAA / GGTGGCCAGCCATACGCT	Cloning *vx3* promoter (2123 bp)
Pphr2-F/R	ATACTGGGGACAGACACTTGC / GTTTCCTGATTCGAAACTGGA	Cloning *phr2* promoter (1904 bp)
PcnB-F/R	AAGGGGACTGGGTAAAGCTG / CGGAGAGCGAGCGACAG	Cloning *cnB* promoter (1571 bp)
bdCla4A-F/R	ATGGCCATGGAGGCC**GAATTC**ATGTCACAGAACAGCGTATA / CGCTGCAGGTCGAC **GGATCC**TTATTTGGCATGCTTCTTGA	Cloning *cla4A* cDNA (2550 bp) for ligation to BD
adCdc24-F/R	GCCATGGAGGCCAGT**GAATTC**ATGAAGAGCGCCCCTCGAAG / CAGCTCGAGCTCG ATGGATCCCTAGGAAACGTCACCCACGC	Cloning *cdc24* cDNA (2913 bp) for ligation to AD
adCdc42-F/R	GCCATGGAGGCCAGT**GAATTC**ATGGCCGTCGTCGCAACCAT / CAGCTCGAGCTCGA T**GGATCC**TTACAAAATGCGACATTTGT	Cloning *cdc42* cDNA (585 bp) for ligation to AD
adSte11-F/R	GCCATGGAGGCCAGT**GAATTC**ATGCTGGCCACCAAGCCGCC / CAGCTCGAGCTCG ATGGATCCTCACGTGATGGGGTTGAGAA	Cloning *ste11* cDNA (2727 bp) for ligation to AD
adSte20-F/R	GCCATGGAGGCCAGT**GAATTC**ATGGACAAGAATTCGTCCCA / CAGCTCGAGCTCG ATGGATCCTCACGCTTTGCGCAACTTTT	Cloning *ste20* cDNA (2640 bp) for ligation to AD
adFpr3-F/R	GCCATGGAGGCCAGT**GAATTC**ATGGCTGGCATCTCTGGTCC / CAGCTCGAGCTCGA T**GGATCC**CTTGATCTCGAGCAGCTTGA	Cloning *fpr3* cDNA (1389 bp) for ligation to AD

* Underlined regions are DNA fragments to exchange for the corresponding fragments of vectors constructed at the sites (in bold) of restriction enzymes for *cla4A* fusion to N-terminus of *GFP* (*Bam*HI/*Xma*I), homogenous recombination of *bar*-separated 5′ and 3′ fragments for *cla4A* or *cla4B* deletion (*Bam*HI/*Xma*I and *Xba*I/*Hpa*I), gateway exchange fragment for targeted gene complementation (*Hin*dIV/*Xba*I), or ligation of targeted gene to BD or AD (*EcoR*I/*BamH*I) for Y2H assays.