Supplemental Material

Two photolyases repair distinct DNA lesions and reactivate UVB-inactivated conidia of an insect mycopathogen under visible light

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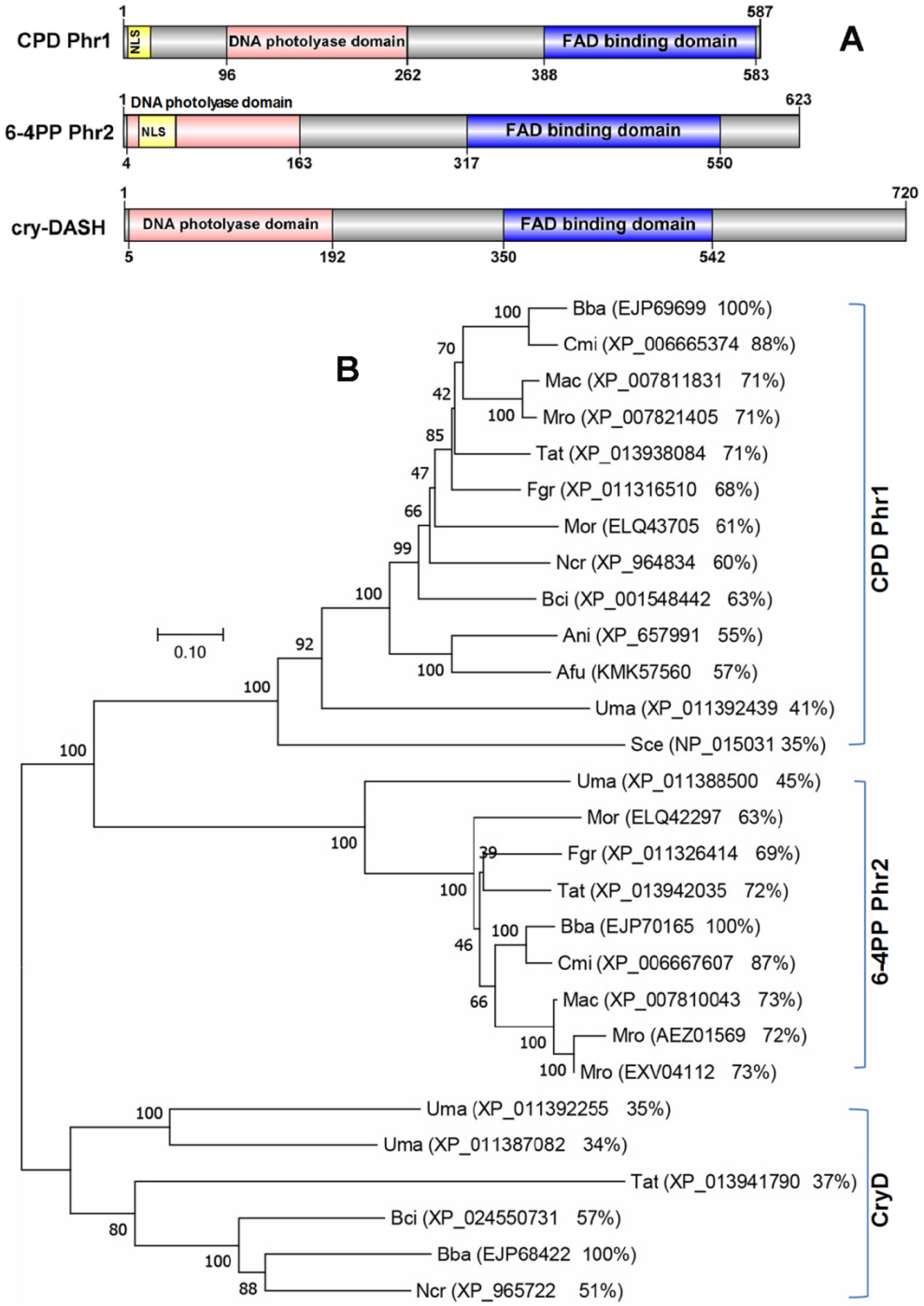

FIG S1 Bioinformatic analysis of cryptochrome/photolyase family members in selected fungi. (**A**) Structural features of CPD Phr1, 6-4PP Phr2 and CryD (cry-DASH) in *B. bassiana*. (**B**) Phylogenetic linkages of *B. bassiana* Phr1, Phr2 and CryD with the homologs of other entomopathogenic and non-enntomopathogenic fungi. The NCBI accession code of each protein and the identity of its sequence to the *B. bassiana* (Bba) counterpart are given in the parentheses following the abbreviated fungal name (Afu, *Aspergillus fumigatus*; Ani, *A. nidulans*; Bci, *Botrytis cinereal*; Cmi, *Cordyceps militaris*; Fgr, *Fusarium graminearum*; Mac, *Metarhizium acridum*; Mor, *Magnaporthe oryzae*; Mro, *Metarhizium. robertsii*; Ncr, *Neurospor crassa*; Sce, *Saccharomyces cerevisiae*; Tat, *Trichoderma atroviride*; Uma, *Ustilago maydis*). The bootstrap values of 1000 replications are given at nodes. Scale: branch length proportional to genetic distance assessed with the neighbor-joining method in MEGA7 software at http://www.megasoftware.net.

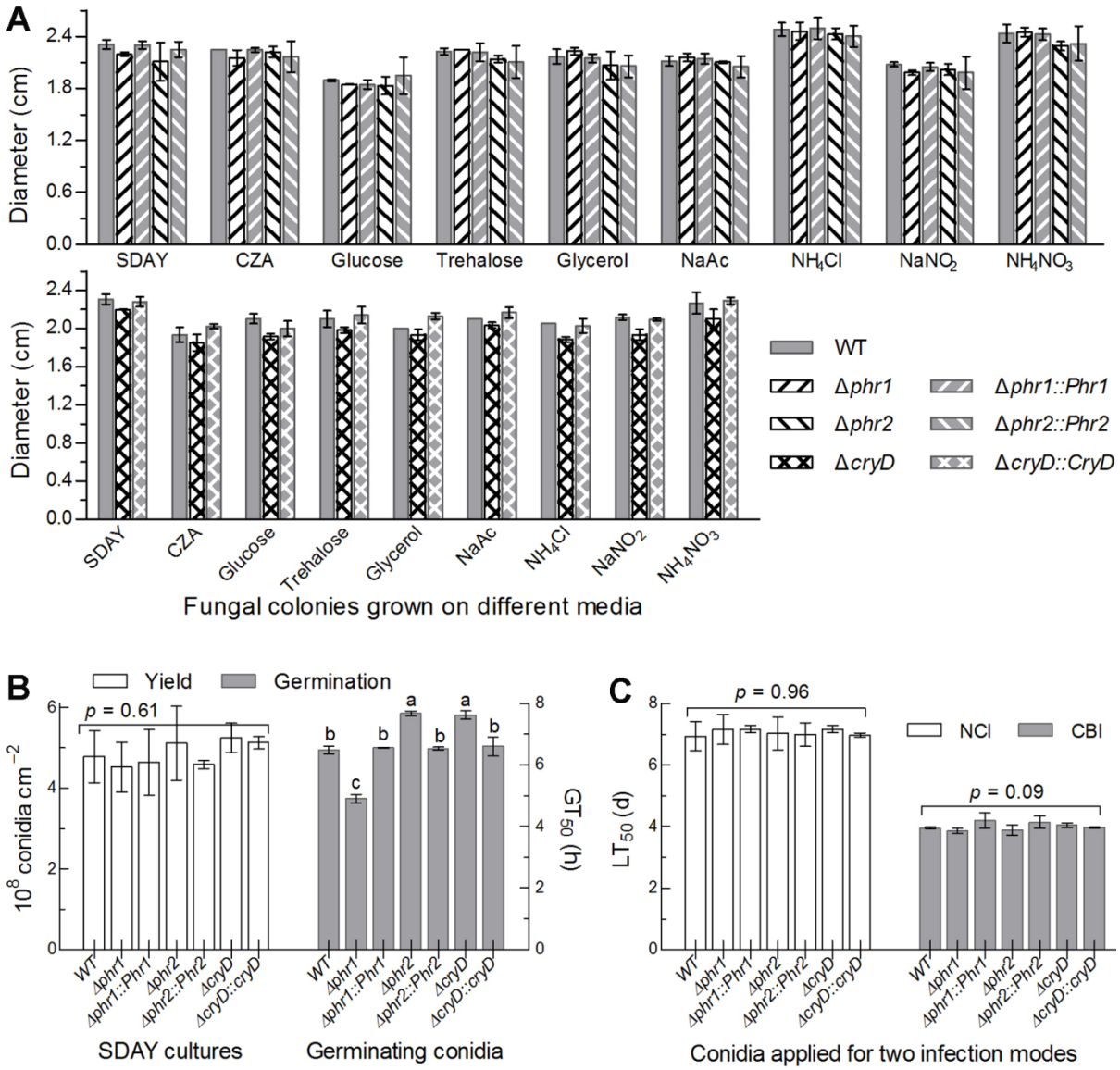

FIG S2 Singular deletions of *Phr1*, *Phr2* and *CryD* in *B. bassiana* affect conidial germination but are not influential on growth rates, conidiation capacity and virulence. (**A**) Diameters of fungal colonies after 8 days of incubation at 25°C and L:D 12:12 on rich SDAY, minimal CDA and modifed CDAs with different carbon or nitrogen sources. Each colony was initiated with 1 μl of a 10^6 conidia ml^{-1} suspension. (**B**) Conidial yields in the 8-day-old SDAY cultures, which were initiated by spreading 100 μl of a 10^6 conidia ml^{-1} suspension per plate and incubated at 25°C and L:D 12:12, and GT_{50} estimates for 50% germination of collected conidia at 25°C. (**C**) LT_{50} estimates for the virulence of deletion mutants and control strains to *G. mellonella* larvae, which were inoculated by topical application (immersion) of a 10^7 conidia ml^{-1} suspension for normal cuticle infection (NCI) or intrahemocoel injection of 5 μl of a 10^5 conidia ml^{-1} suspension per larva for cuticle-bypassing infection (CBI). The *p* values indicate no significant variability for the examined phenotypes of all strains. Different lowercase letters represent significant differences (Tukey's HSD, $p < 0.05$). Error bars: SD from three replicates.

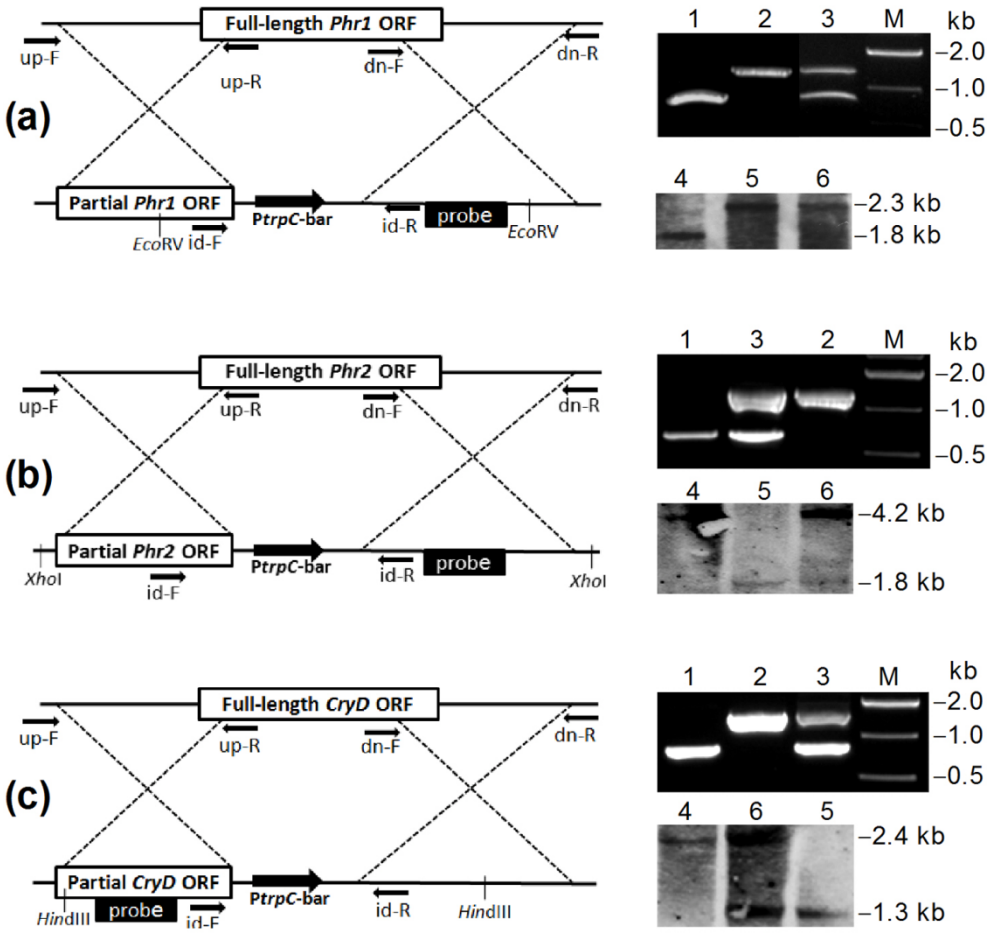

FIG S3 Schematic diagram for the deletion strategy of *phr1* (**A**), *phr2* (**B**) and *cryD* (**C**) in *B. bassiana* and their mutants identified through PCR (lanes 1–3) and Southern blot (lanes 4–6) analyses with paired primers and amplified probes (Table S1). Lanes 1 and 4: wild-type. Lanes 2 and 5: deletion mutants. Lanes 3 and 6: complementary mutants. Genomic DNA of each strain was digested with *Eco*RV, *Xho*I and *Hin*dIII at the marked sites to probe *phr1*, *phr2* and *cryD* through Southern blotting hybridization, respectively.

Table S1. Paired primers designed for manipulation of *Phr1*, *Phr2* and *CryD* in *B. bassiana*.

Primers	Paired sequences (5′-3′)*	Purpose
Ptef-F/R	CCACCATGTTGGGCCCGGCGCGCCTACTGCCGCAAGCAATTCTTTA / CAGGTCGACGGATCCCCGGGTTTGAAGGTGTTTGTGAT	Cloning *Tef* promoter (1369 bp)
cPhr1-F/R	CAATCACAAACACCTTCAAAATGGCGCCTCGTGCAACGAAGCG / CTTGCTCACCATGAATTCGATATCCTACGCCACAGCCGCCTTGTACGCA	Cloning *phr1* cDNA (1764 bp) for fusion with *GFP*
cPhr2-F/R	CAATCACAAACACCTTCAAAATGACAAAGCCCAGAGTCATTTATT / CTTGCTCACCATGAATTCGATATCTCACGTTTTCTGCTTCTTCGCTGAC	Cloning *phr2* cDNA (1872 bp) for fusion with *GFP*
cCryD-F/R	CAATCACAAACACCTTCAAAATGACTGCCAGCAGAGTTCTCGTCT / CTTGCTCACCATGAATTCGATATCGTTGGGCATATACCCTCCTCTGCCT	Cloning *cryD* cDNA (2163 bp) for fusion with *GFP*
Phr1up-F/R	TGGGCCCGGCGCGCCGAATTCTCTTGGAAGCGACGGTGTA / TGGCTGCAGGTCGACGGATCCTGGCGAGTTGTCTGGTGA	Cloning *phr1* 5′-end (1428 bp) for *Phr1* deletion
Phr1dn-F/R	GACCCATGGCTCGAGTCTAGAGACTTCGAGGCTCATCTCA /GTGGCTAGCGTTAACACTAGTAACAATAGTCTGCTGGTGGA	Cloning *phr1* 3′-end (1532 bp) for *phr1* deletion
Phr2up-F/R	TGGGCCCGGCGCGCCGAATTCCAGAAGCCAGCTCCATTC / TGGCTGCAGGTCGACGGATCCACGGCACTTTGTCTCAGTAG	Cloning *phr2* 5′-end (1458 bp) for *phr2* deletion
Phr2dn-F/R	GACCCATGGCTCGAGTCTAGACCGCGTCCATCACCAAGCT / GGTGGTGGTGGCTAGCGTTAACGCCGCAAGGCAAATCTCG	Cloning *phr2* 3′-end (1447 bp) for *phr2* deletion
CryDup-F/R	TGGGCCCGGCGCGCCGAATTCAGTTGAACATTATTGTGGGTAC / TGGCTGCAGGTCGACGGATCCGAGTTGAAGGAGGAGAACC	Cloning *cryD* 5′-end (1477 bp) for *cryD* deletion
CryDdn-F/R	GACCCATGGCTCGAGTCTAGAATACCCTTTCTTCCCACCA / GTGGCTAGCGTTAACACTAGTATTGCCAATTAGCCCAGT	Cloning *cryD* 3′-end (1235 bp) for *cryD* deletion
Phr1fl-F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTTTGTAATGGCAATAGATG / GGGGACCACTTTGTACAAGAAAGCTGGGTTGTTGACCGATTCGTAGATG	Cloning full-length *phr1* (4616 bp) for *phr1* complementation
Phr2fl-F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCTCATCTGGGAAATGAAAGGA / GGGGACCACTTTGTACAAGAAAGCTGGGTCCAGCCGAGTATTACGAT	Cloning full-length *phr2* (5636 bp) for *phr2* complementation
CryDfl-F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAGTATCTGCGGTGAGC / GGGGACCACTTTGTACAAGAAAGCTGGGTAGCCGTGATTTCTTCTGC	Cloning full-length *cryD* (5678 bp) for *cryD* complementation
pPhr1-F/R	AAAAAGAGAATCCGTCTTCACTTACA / AAAAAGCCAGTAGCCTTATTATCTTG	PCR detecting *phr1*
pPhr2-F/R	AAAAAGTTCACATCATTTCTCCACCAC / AAAAAGCTTCGACCGCAGCATCT	PCR detecting *phr2*
pCryD-F/R	AAAAAAGATTCGGTTCGCTTCGT / AAAAAAAGGCACATCGTCAGTCATC	PCR detecting *pryD*
sbPhr1-F/R	CTGTCGGCGGAAGAAC / GACTAATACGTGGCGATAAA	Southern probe of *phr1* (302 bp)
sbPhr2-F/R	TACAGCCCCGTCTCGT / CCGCAAGGCAAATCTC	Southern probe of *phr2* (250 bp)
sbCryD-F/R	CGCTATCAAGGGTCCAAA/GTGACGGGCTCGACAAAG	Southern probe of *pryD* (284 bp)
qActin-F/R	GGCAACATTGTCATGTCTGG/TTTGCTGGAAGGTGGATAGG	qPCR detecting *β-actin*
qPhr1-F/R	CACCGCAAGAGACAGAAACA/AGCGTTAAAGTGCTCGGTGT	qPCR detecting *phr1*
qPhr2-F/R	TCATTTATTGGTTCCGCACA/GCTTGGATTTGGAGTTGAGC	qPCR detecting *phr2*
qCryD-F/R	CGTATTACCTCCCGACCAGA/GCACTGCAGATGCTGGATAA	qPCR detecting *cryD*
OEphr1-F/R	CAAACACCTTCAAACCCGGGATGGCGCCTCGTGCAACGAAGCG / GGCTGCAGGTCGACGGATCCCTACGCCACAGCCGCCTTGTACGCA	Cloning *phr1* cDNA (1764 bp) for overexpression
OEphr2-F/R	CAAACACCTTCAAACCCGGGATGACAAAGCCCAGAGTCATTTATT/GGCTGCAGGTCGACGGATCCTCACGTTTTCTGCTTCTTCGCTGAC	Cloning *phr2* cDNA (1872 bp) for overexpression
pOEphr1-F/R	TACTGCCGCAAGCAATTCTTTA / CTACGCCACAGCCGCCTTGTACGCA	PCR detecting OE*Phr1*
pOEphr2-F/R	TACTGCCGCAAGCAATTCTTTA / TCACGTTTTCTGCTTCTTCGCTGAC	PCR detecting OE*Phr2*

* Blue regions denote the restriction enzyme sites for the deletion of *phr1* (*Eco*RI/*Bam*HI and *XbaI*/*SpeI*), *phr2* (*Eco*RI/*Bam*HI and *Xba*I/*Hpa*I) or *cryD* (*Eco*RI/*Bam*HI and *Xba*I/*Spe*I), or for the fusion of *phr1*, *phr2* or *cryD* cDNA (*Eco*RV) to *GFP*. Underlined regions denote the fragments for the fusion of *phr1*, *phr2* or *cryD* to *GFP*, the targeted gene deletion, or the gateway exchange for targeted gene complementation or overexpression.