

This file contains supplementary data for the following paper submitted to
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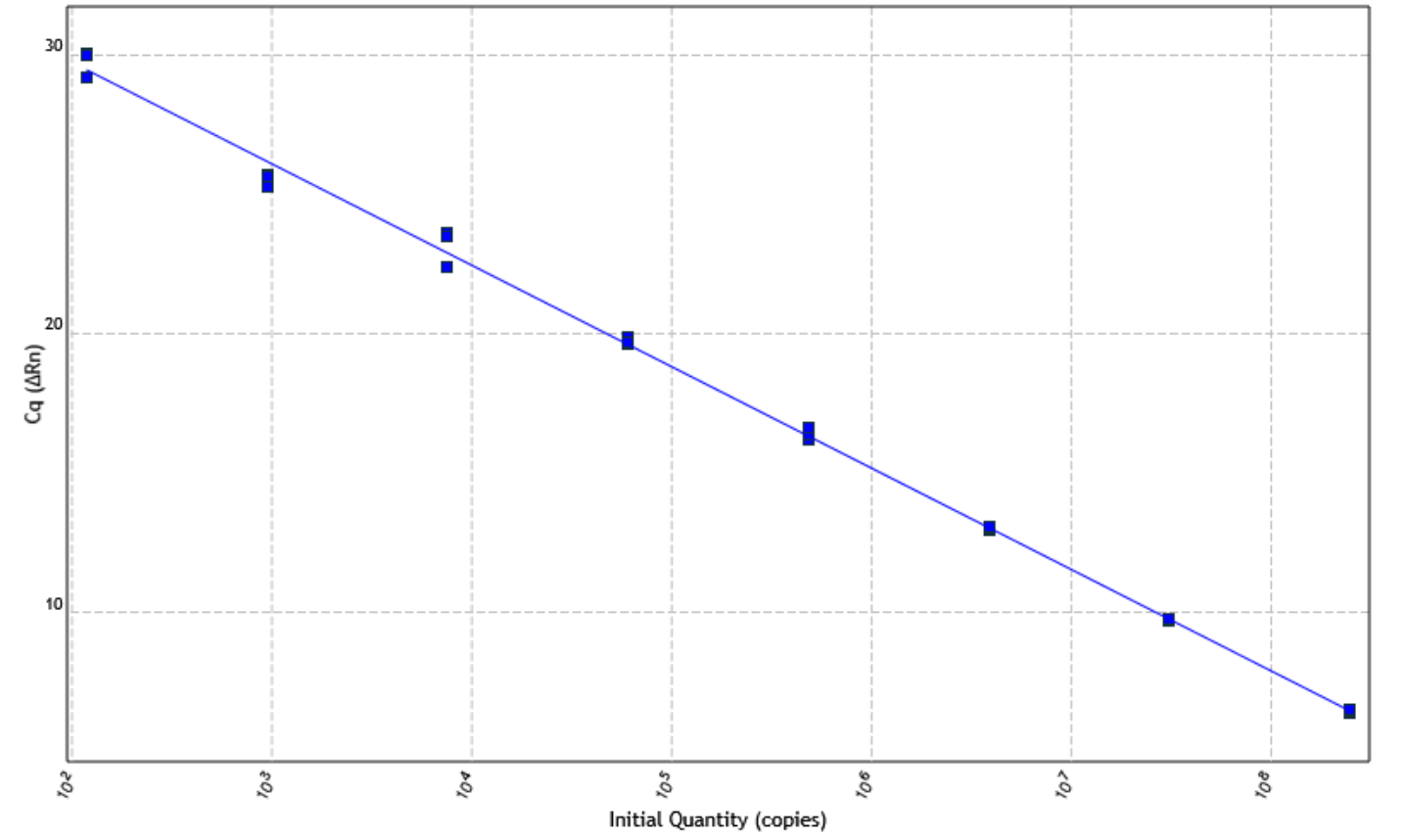
In vivo transcriptome analysis of the entomopathogenic fungus *Beauveria bassiana* strain GHA during infection against the beneficial predator tobacco leaf bug, *Nesidiocoris tenuis* (Hemiptera: Miridae)

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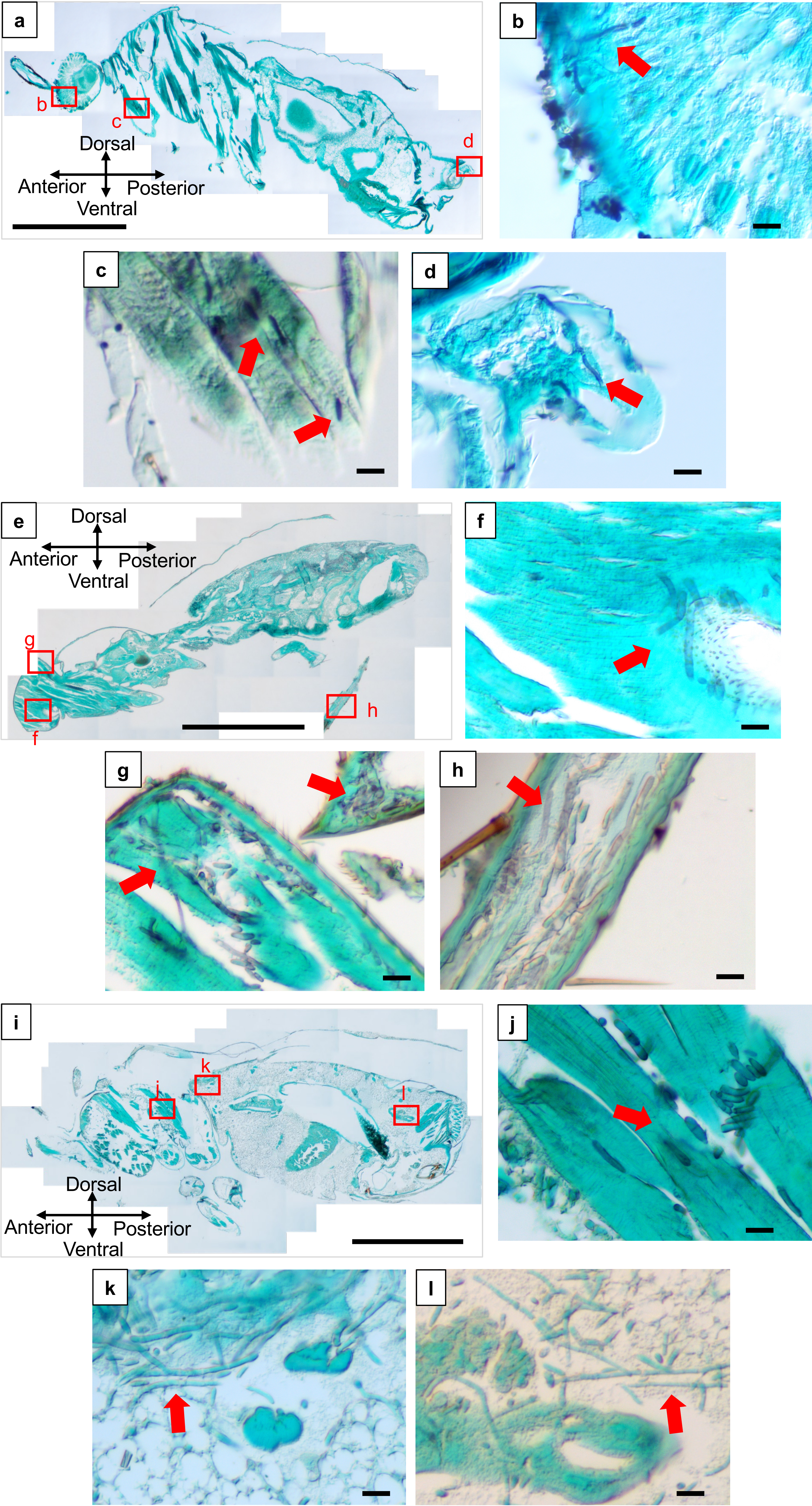
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Supplementary Figure S1



Supplementary Fig. S1 A standard curve prepared for the absolute quantification of genomic DNA from *Beauveria bassiana* strain GHA using qPCR. The vertical axis (Cq: quantification cycle) represents the number of cycles required for the qPCR sample to reach a fluorescence intensity of $\Delta Rn = 0.1$. The horizontal axis represents copy number of the ribosomal DNA intergenic spacer (rIGS) region. The 2,097-bp PCR amplicon containing the rIGS region of *B. bassiana* strain GHA (the sequence of 2,057 bp, excluding the primer sequences, is registered under accession number LC939607) was amplified from genomic DNA of strain GHA with a primer pair consisting of GHArIGS_F1 and GHArIGS_R1, purified, and used as a template for qPCR. The copy number of rIGS in the template was calculated based on the nucleic acid concentration ($\text{ng}/\mu\text{L}$) and molecular weight ($1.29565783 \times 10^6 \text{ g/mol}$) of the amplicon, and Avogadro constant N_A ($= 6.02214076 \times 10^{23} \text{ mol}^{-1}$). An 8-fold dilution series of the rIGS amplification products ranging from 1.13×10^2 to 2.38×10^8 copies/well was used as as templates.

Supplementary Figure S2



Supplementary Fig. S2 Cross section of tobacco leaf bug inoculated with strain GHA at 36 hpi (a–d), 48 hpi (e–h), and 72 hpi (i–l). **a, e, i:** Whole body, **b:** Head, **c, f, g, j:** Thorax, **d:** Abdominal extremity, **h:** Leg, **k:** Abdominal back, **l:** Abdominal cavity. Scale bar = 500 μm (a, e, i), 10 μm (b–d, f–h, j–l). The red arrows indicate hyphae and hyphal bodies.

Supplementary Table S1. List of enrich GO terms expressed at cuticle-penetration phase (36 h post inoculation).

GO Category	Term ID	Term description	Observed / Background gene count	Fold Enrichment	Enrichment FDR
Biological Process	GO:0022409	Pos. reg. of cell-cell adhesion	2/3	104.28	4.07.E-03
	GO:0043434	Response to peptide hormone	2/3	104.28	4.07.E-03
	GO:0034097	Response to cytokine	3/7	67.04	1.30.E-03
	GO:0071345	Cellular response to cytokine stimulus	3/7	67.04	1.30.E-03
	GO:0007166	Cell surface receptor signaling pathway	2/5	62.57	9.32.E-03
	GO:0022407	Reg. of cell-cell adhesion	2/5	62.57	9.32.E-03
	GO:0042127	Reg. of cell population proliferation	2/5	62.57	9.32.E-03
	GO:0009725	Response to hormone	3/11	42.66	3.00.E-03
	GO:0002376	Immune system process	3/14	33.52	4.07.E-03
	GO:0051239	Reg. of multicellular organismal process	3/15	31.28	4.07.E-03
	GO:0009719	Response to endogenous stimulus	3/33	14.22	2.25.E-02
Cellular Component	GO:0005576	Extracellular region	20/429	6.21	5.88.E-10
Molecular Function	GO:0042973	Glucan endo-1,3-beta-D-glucosidase activity	2/7	43.77	2.75.E-02
	GO:0008422	Beta-glucosidase activity	3/24	19.15	2.75.E-02
	GO:0005102	Signaling receptor binding	3/27	17.02	2.75.E-02
	GO:0015926	Glucosidase activity	3/36	12.77	4.28.E-02
	GO:0008233	Peptidase activity	8/305	4.02	2.75.E-02
	GO:0016787	Hydrolase activity	21/1549	2.08	2.75.E-02

Supplementary Table S2. Transcriptome profiles of housekeeping genes in the strain GHA

Genbank ID	Gene annotation	Fold change	<i>p</i> -value	Raw read count	
		72 h / 36 h		36 h	72 h
XM_008599957.1	Actin-like protein	1.84	0.52	34	1670
XM_008601341.1	Beta tubulin	1.04	0.97	29	806
XM_008596666.1	Elongation factor 1-beta	1.79	0.54	38	1814
XM_008602257.1	Elongation factor 1-gamma	1.67	0.58	55	2440
XM_008596801.1	Elongation factor 2	1.43	0.70	120	4557
XM_008597485.1	Translation elongation factor 1 alpha	1.51	0.65	470	18892
XM_008600577.1	glyceraldehyde-3-phosphate dehydrogenase	0.98	0.76	55	1943
XM_008595305.1	40S ribosomal protein S2	0.94	0.98	91	2374
XM_008599148.1	40S ribosomal protein S7	0.98	0.95	63	1580
XM_008600613.1	40S ribosomal protein S8	1.20	0.98	79	2060
XM_008602826.1	40S ribosomal protein S9	1.43	0.84	92	2947
XM_008596854.1	40S ribosomal protein S11	1.19	0.70	71	2701
XM_008596195.1	40S ribosomal S12 protein	1.11	0.85	64	2027
XM_008596305.1	40S ribosomal protein S13	1.24	0.91	63	1869
XM_008598356.1	40S ribosomal protein S14 (CRP2)	1.71	0.82	49	1623
XM_008599323.1	40S ribosomal protein S15	1.07	0.57	81	3690
XM_008602659.1	40S ribosomal protein S17	1.37	0.95	58	1646
XM_008603455.1	40S ribosomal protein S18	0.90	0.74	102	3707
XM_008599258.1	40S ribosomal protein S19	1.98	0.91	34	812
XM_008595506.1	40s ribosomal protein s20	1.15	0.46	66	3474
XM_008596760.1	40S ribosomal protein S21 (CRP7)	1.37	0.88	72	2210
XM_008596196.1	40S ribosomal protein S22	1.39	0.74	64	2330
XM_008598442.1	40S ribosomal protein S24	1.20	0.73	72	2660
XM_008598284.1	40S ribosomal protein S27	2.67	0.85	56	1787
XM_008597466.1	40S ribosomal protein S28	1.58	0.31	20	1424

XM_008602968.1	40S ribosomal protein S30, putative	1.19	0.63	46	1932
XM_008595271.1	60S acidic ribosomal protein P0	1.41	0.85	82	2602
XM_008598422.1	60S acidic ribosomal protein P1	1.11	0.72	52	1956
XM_008602134.1	60S acidic ribosomal protein P2, putative	1.24	0.91	75	2224
XM_008603130.1	60S ribosomal protein	1.30	0.82	39	1288
XM_008599176.1	60S ribosomal protein L2	1.52	0.78	122	4230
XM_008604017.1	60S ribosomal protein L3	1.20	0.65	126	5113
XM_008599978.1	60S ribosomal protein L4	1.25	0.86	42	1337
XM_008601242.1	60S ribosomal protein L5 (CPR4)	1.76	0.81	90	3002
XM_008598355.1	60S ribosomal protein L7	0.97	0.55	57	2665
XM_008600512.1	60S ribosomal protein L10-A-like protein	1.34	0.98	89	2301
XM_008595252.1	60S ribosomal protein L11	1.53	0.76	48	1709
XM_008602358.1	60S ribosomal protein L12	1.07	0.65	108	4410
XM_008600791.1	60S ribosomal protein L13	1.24	0.94	84	2398
XM_008604751.1	60S ribosomal protein L14-B	1.63	0.82	98	3246
XM_008598297.1	60S ribosomal protein L15	1.29	0.60	56	2433
XM_008604275.1	60S ribosomal protein L16	1.23	0.80	37	1266
XM_008596768.1	60S ribosomal protein L23	1.91	0.82	46	1511
XM_008601502.1	60S ribosomal protein L23a	1.08	0.49	58	2948
XM_008601843.1	60S ribosomal protein L24	1.26	0.94	52	1497
XM_008603651.1	60S ribosomal protein L27	0.87	0.81	61	2043
XM_008595756.1	60S ribosomal protein L28	1.47	0.88	78	1810
XM_008600511.1	60S ribosomal protein L30	1.06	0.68	61	2380
XM_008595314.1	60S ribosomal protein L31	1.96	0.96	65	1826
XM_008598852.1	60S ribosomal protein L32	1.05	0.48	27	1408
XM_008603149.1	60S ribosomal protein L33	1.28	0.96	55	1537
XM_008601755.1	60S ribosomal protein L37	1.35	0.79	44	1501
XM_008599193.1	60S ribosomal protein L39	1.05	0.75	47	1690
XM_008596139.1	60S ribosomal protein L43	1.45	0.96	62	1736

XM_008596930.1	60S ribosomal protein L44 (L41)	1.33	0.69	50	1934
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Supplementary Table S3. List of differentially expressed genes of strain GHA on tobacco leaf bugs whose orthologues has previously been reported as virulence genes in pathogenic fungi

Reference gene accession	Gene	Protein annotation / Related phenotypes other than virulence	Raw read counts		Fold changes ^a	Log ₂ (Fold changes) (72 h / 36 h)	<i>p</i> -value	Reference ^b
			36 h	72 h				
Genes expressed higher at 36 h post inoculation								
XM_008603502.1	<i>cfp</i>	cysteine-free protein / conidial coat formation, multiple stress tolerance (<i>Beauveria bassiana</i>)	13	0	1437.26	-10.49	8.93E-04	Mou et al. (2021)
XM_008597218.1	<i>Bbnmo5</i>	Oxidoreductase, 2-nitropropane dioxygenase family / conidia and blastospore production, cell wall development (<i>B. bassiana</i>)	5	0	553.41	-9.11	1.37E-02	Zhang et al. (2025)
XM_008596755.1	<i>GasI</i>	Cell surface protein / Appressoria formation (<i>Magraporthe grisea</i>)	14	1	300.52	-8.23	6.39E-07	Xue et al. (2002) Zhao et al. (2023)
XM_008597516.1	<i>Mad1, Adh1</i>	GPI-anchor adhesin / adherence to insect cuticle (<i>Metarhizium robertsii</i>)	23	8	74.33	-6.22	3.34E-10	Wang and St. Leger (2007a); Zhou et al. 2021
XM_008595416.1	<i>BpS8</i>	Peptidase S8 family / degrade PPOs (<i>B. bassiana</i>)	6	4	37.72	-5.24	9.25E-04	Zhang et al. (2017)
XM_008603023.1	<i>BbNoxA</i>	NADPH oxidase / germination, conidial production (<i>B. bassiana</i>)	9	10	23.42	-4.55	2.51E-05	Zhu et al. (2024)
XM_008601846.1	<i>ChiV4</i>	Chitinase / Degradation of insect cuticles (<i>B. bassiana</i>)	8	19	11.08	-3.47	9.64E-04	Qiu et al. (2022)
Genes expressed higher at 72 h post inoculation								
XM_008603280.1	<i>OpS5</i>	Oxidoreductase / Oosporein synthesis, detoxification of ROS, interference with phenoloxidase, surface protein masking	0	1470	6101.88	12.58	2.50E-04	Feng et al. (2015); Lu et al. (2021)

		pathogen associated molecular patterns (<i>B. bassiana</i>)							
XM_008603281.1	<i>OpS6</i>	Glutathione S-transferase-like protein / Oosporein synthesis (<i>B. bassiana</i>)	0	788	3271.41	11.68	6.55E-04	Feng et al. (2015)	
XM_008603279.1	<i>OpS4</i>	Hydroxylase / Oosporein synthesis (<i>B. bassiana</i>)	5	518	2150.84	11.07	1.88E-03	Feng et al. (2015)	
XM_008603282.1	<i>OpS7</i>	Oxidoreductase / Oosporein synthesis (<i>B. bassiana</i>)	0	464	1926.72	10.91	2.43E-03	Feng et al. (2015)	
XM_008603276.1	<i>OpS1</i>	Orsellinic acid synthase / Oosporein synthesis (<i>B. bassiana</i>)	0	410	1702.61	10.73	3.33E-03	Feng et al. (2015)	
XM_008603277.1	<i>OpS2</i>	Membrane transporter / Oosporein synthesis (<i>B. bassiana</i>)	0	277	1150.62	10.17	8.53E-03	Feng et al. (2015)	
XM_008596270.1	<i>BbPex1</i>	Peroxisomal ATPase PEX1 (Peroxin-1) / oxidative and osmotic stress tolerance, production of conidia and blastospore, assimilation of nutrients (fatty acid, protein, and chitin) (<i>B. bassiana</i>)	0	140	582.04	9.18	3.74E-02	Hou et al. (2022)	
XM_008603856.1	<i>Mpl1</i>	Perilipin MPL1-like protein / lipid metabolism during appressorial differentiation (<i>M. robertsii</i>)	0	134	557.13	9.12	4.37E-02	Wang and St. Leger (2007b)	
XM_008599131.1	<i>BbHCR1</i>	Putative Zn(II)2Cys6 transcription factor / yeast-to-hyphal morphological switch in insect hemocoel (<i>B. bassiana</i>)	1	364	13.56	3.76	4.16E-02	Deng et al. (2024)	

^a Ratio of high expression conditions to low expression conditions

^b The information for Lu et al. (2021) is provided below. For other literature, please refer to the main text.

Lu Z, Deng J, Wang H, Zhao X, Luo Z, Yu C, Zhang Y (2021) Multifunctional role of a fungal pathogen-secreted laccase 2 in evasion of insect immune defense. Environ Microbiol, 23(2):1256–1274. <https://doi.org/10.1111/1462-2920.15378>