

Appendix B

Parasitoid evenomation alters the *Galleria mellonella* midgut microbiota and immunity, thereby promoting fungal infection

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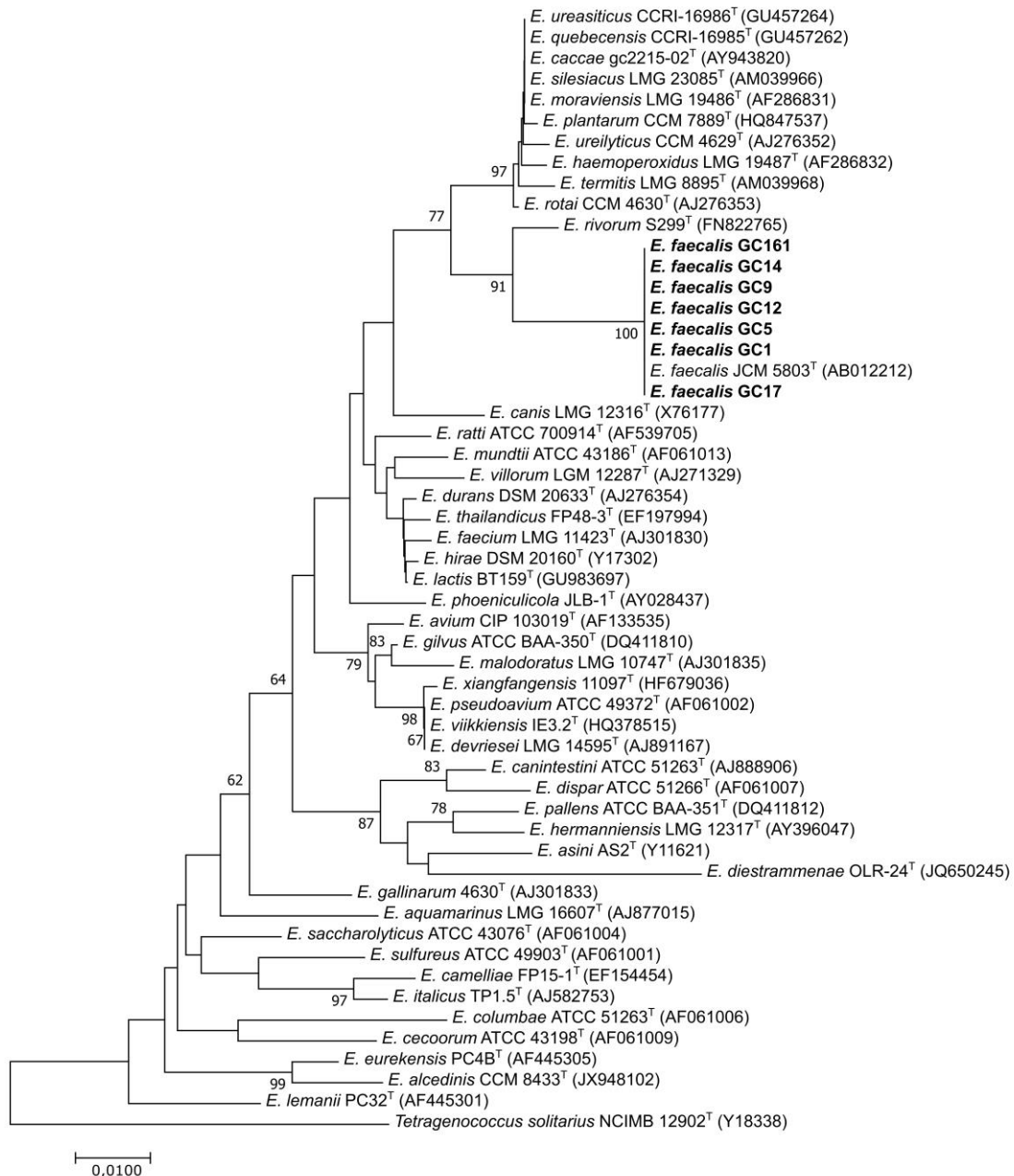


Figure S1. Phylogenetic analysis of the investigated 16S rRNA gene sequences of cultivable *Enterococcus* strains from *Galleria mellonella* midguts, along with the most similar sequences. The GenBank identifiers (gi) for the sequences are shown in parentheses. The neighbor-joining method and the maximum composite likelihood model were used to construct the trees. Bootstrap statistical support (1000 replicates) of greater than 60% is shown at the nodes. The sequence IDs of the investigated bacterial strains are in bold font.

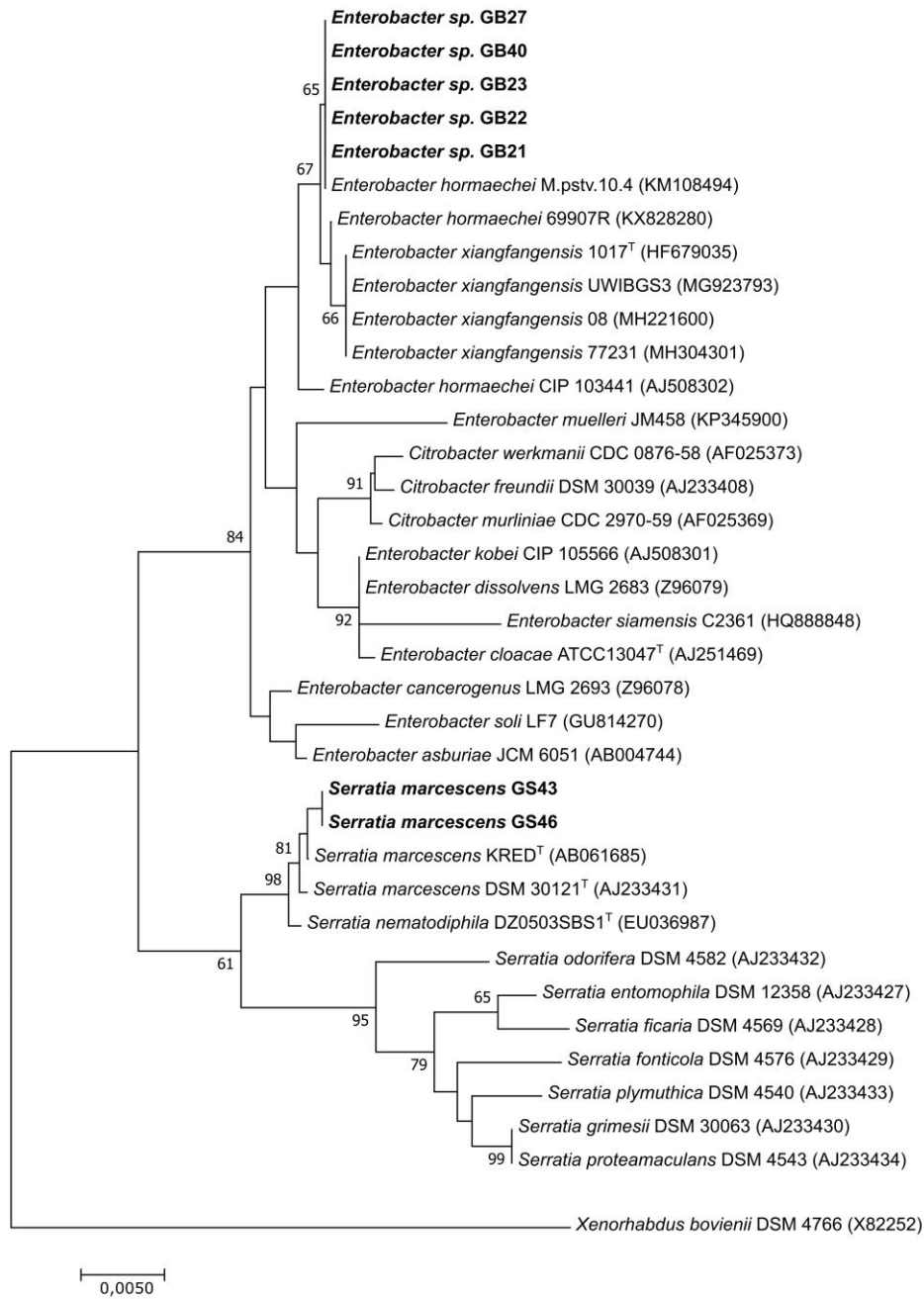


Figure S2. Phylogenetic analysis of the investigated 16S rRNA gene sequences of cultivable *Enterobacter* and *Serratia* strains from *Galleria mellonella* midguts, along with the most similar sequences. The GenBank identifiers (gi) for the sequences are shown in parentheses. The neighbor-joining method and the maximum composite likelihood model were used to construct the trees. Bootstrap statistical support (1000 replicates) of greater than 60% is shown at the nodes. The sequence IDs of the investigated bacterial strains are in bold font.

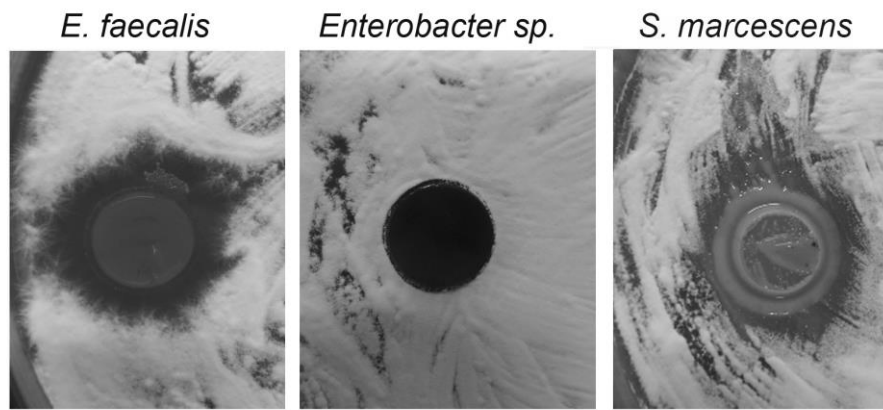


Figure S3. Inhibition of *Beauveria bassiana* mycelial growth by bacteria isolated from wax moth midguts. The diameter of the plugs is 8 mm.

Table S1. Expected and observed mortality of wax moth larvae after treatment with *B. bassiana* (Bb) and cultivable midgut bacteria.

	Days after treatment									
	1	2	3	4	5	6	7	8	9	10
Bb and Entrococcus										
Expected mortality, %	0	1,39	7,65	15,3	20,7	35	38,1	42,1	47,4	52,1
Observed mortality, %	0,67	1,33	24,1	35,4	42,7	54,7	66,2	67,8	73,5	77,3
Observed – Expected	0,67	-0,1	16,4	20,1	22	19,8	28,1	25,7	26,1	25,2
χ^2	–	0	58,6*	48	45,3	26,4	51,7	41,6	42,2	39,1
Bb and Entrobacter										
Expected mortality, %	0	1,39	7,65	15,3	20,7	35	38,1	42,1	47,4	52,1
Observed mortality, %	0	0	20,7	32,5	39,8	53,3	64	68,8	70,9	75,2
Observed – Expected	0	-1,4	13,1	17,1	19,1	18,3	25,9	26,6	23,5	23
χ^2	–	2,42	41,7	38,9	38	25,3	49,1	50,1	38,1	36,6
Bb and Serratia										
Expected mortality, %	0	0,63	6,09	14,9	20,3	33,8	36,9	42,1	47,3	51,4
Observed mortality, %	0	0	19,9	28,8	43,1	66,8	74,7	77,1	81,2	83
Observed – Expected	0	-0,6	13,8	14	22,9	33,1	37,8	35	33,9	31,7
χ^2	–	1,12	59,2	27,4	57,5	87	109	89,6	82,2	71,5

* - Values in bold font show significant synergistic effects ($\chi^2 > 3.84$, $df = 1$, $P < 0.05$) when analyzed as described by Robertson and Preisler⁴.

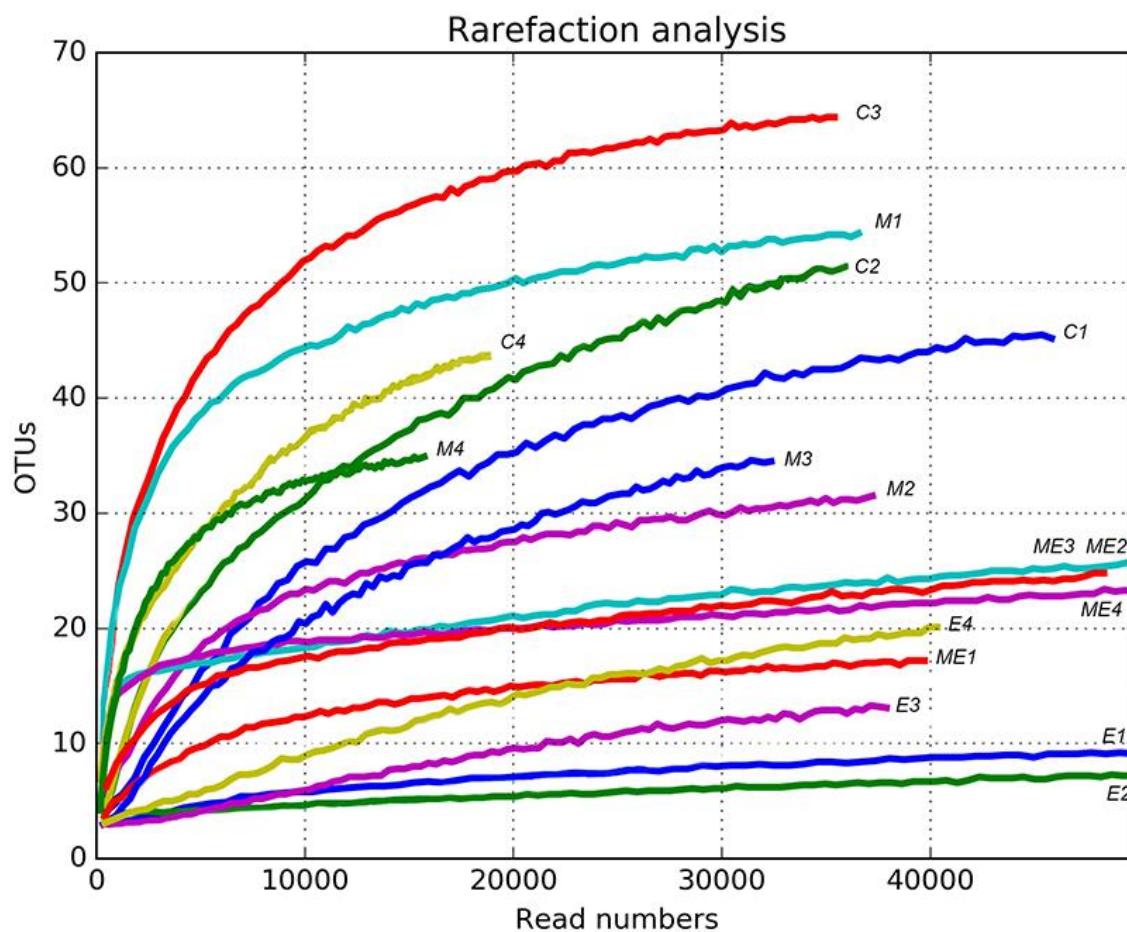


Figure S4. Rarefaction curves of the OTU number for each sample. *C* – control; *M* – mycosis; *E* – envenomation; *EM* – mycosis and envenomation; 1, 2, 3, 4 – replicates.

Table S2. List and description of genes and primers sequences used in the qPCR.

Gene Name	NCBI genebank Accession number	Gene symbol	Primer sequence (5' – 3')	Product size (bp)	PCR efficiency (\pm SD)	Tm in qPCR, °C	Primers source
Translation elongation factor 1-alfa	AF423811.1	EF1a	For CTG(AACCTCCTTACAGTGAATCC)* Rev GC(ATGTTATCTCCGTGCCAG)*	135(127)	2,05 \pm 0,06/ 2,04 \pm 0,02/ (2,02 \pm 0,04)	62/60/(57)	Melo et al., (2013) ^b
11 subunit of eukaryotic DNA-depended RNA polymerase II	NTHM01000029.1 (wgss) GME-string_Contig_515.0 ^a	RBP11	For CGCCAACCTTTGAATCATTCCCTT Rev TGGTGTCTGATCATGTTTCCAAGA	136	1,92 \pm 0,08/ 1,96 \pm 0,04/ 1,93 \pm 0,06	62/60/57	ISEA ^c
Gallerimycin	AF453824.1	Gal	For GAAGTCTACAGAATCACACGA Rev ATCGAAGACATTGACATCCA	161	1,92 \pm 0,4	62	Melo et al., (2013) ^b
Gloverin	AF394588.1	Glo	For AGATGCACGGTCCTACAG Rev GATCGTAGGTGCCTTGTG	93	2,00 \pm 0,06	62	Melo et al., (2013) ^b
Galiomycin	AY528421.1	Glm	For GTGCGACGAATTACACCTC Rev TACTCGCACCAACAATTGAC	103	2,02 \pm 0,03	62	Melo et al., (2013) ^b
Inducible metalloproteinase inhibitor	AY330624.1	IMPI	For TAGTAAGCAGTAGCATAGTCC Rev GCCATCTTCACAGTAGCA	161	2,04 \pm 0,04	57	Melo et al., (2013) ^b
Heat shock protein 90	AF394591.1	Hsp90	For TCAGCTTCACGGACAGCTTCT Rev GACCCCAGAGCTTGCATTGG	152	2,03 \pm 0,05	62	ISEA ^c
Caspase-like protein	NTHM01000022.1 (wgss) contig15362_1 ^a	CASP	For AGCTACATCCCAGAGGATTCA Rev TCTTCTGTGGGGCAGTCA	104	2,01 \pm 0,03	60	ISEA ^c
Glutathione peroxidase	NTHM01000062.1 (wgss) contig17373_1 ^a	GSH PX	For CCACACTGTGAGGCAACATT Rev GTTTGCTTAGCACGGTCACA	188	1,99 \pm 0,07	62	Melo et al., (2013) ^b
Macrophage migration inhibitory factor	NTHM01000005.1 (wgss) contig20582_1 ^a	MIF	For AACAGTACTGCGTGGTGA Rev TTCCACGCCAAGGGATCCAA	108	2,03 \pm 0,04	60	ISEA ^c

* round brackets denote modifications for Tm = 57 °C

^a Vogel and coauthors¹

^b Melo and coauthors²

^c Primers were designed in the Laboratory of Insect Pathology ISEA SB RAS with an online resource, <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>, and their properties were estimated by IDT OligoAnalyzer 3.1 (<http://eu.idtdna.com/calc/analyzer>).

References

1. Vogel, H., Altincicek, B., Glöckner, G. & Velcinskas, A. A comprehensive transcriptome and immune-gene related repertoire of the lepidopteran model host *Galleria mellonella*. *BMC Genomics* **12**, 308, (2011).
2. Melo, N.R., Abdrahman, A., Greig, C., Mukherjee, K., Thornton, C., Ratcliffe, N.A., Vilcinskas, A. & Butt, M.T. Myriocin significantly increases the mortality of a non-mammalian model host during *Candida* pathogenesis. *PlosOne*, 2013. DOI: 10.1371/journal.pone.0078905.
3. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis 7.0 for bigger datasets *Molecular Biology and Evolution* 33:1870-1874.
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