Supplementary Material

Supporting information related to the manuscript: Rapid and sustained differentiation of disease-suppressive phyllosphere microbiomes in tomato following experimental microbiome selection

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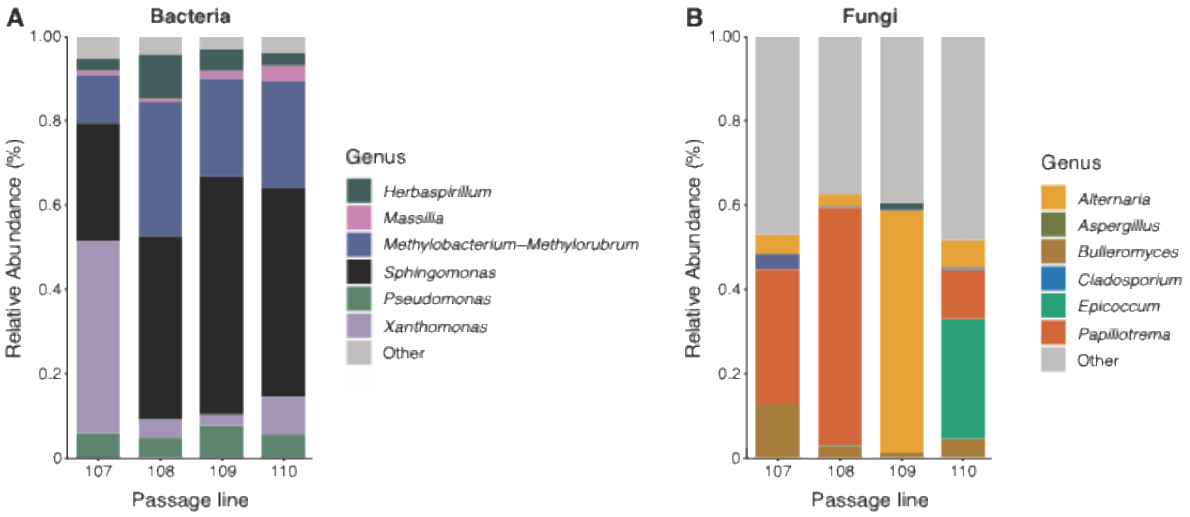
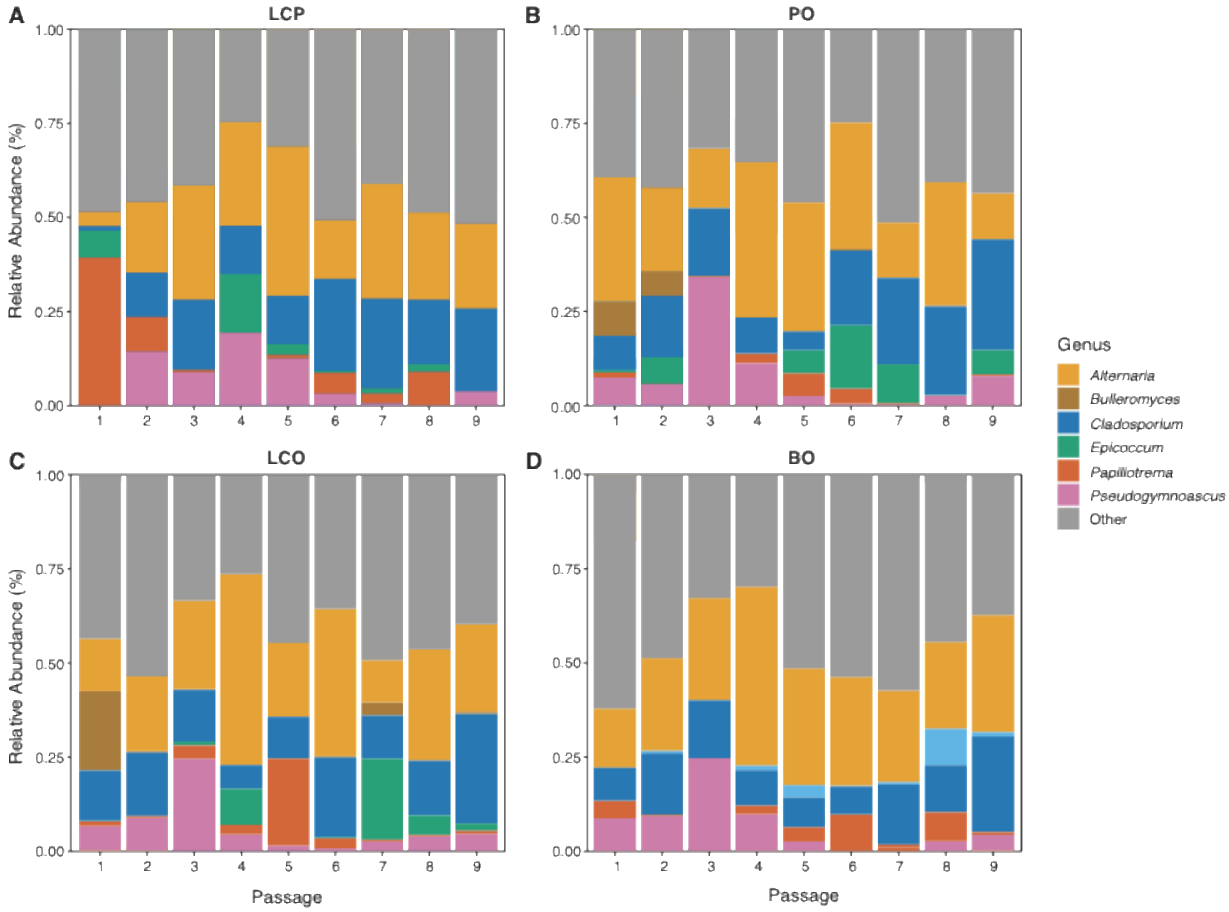


Figure S1. Source composition at the genus level across four passage lines (107, 108, 109, 110) for each treatment. 'Other' includes all taxa assigned <3%.



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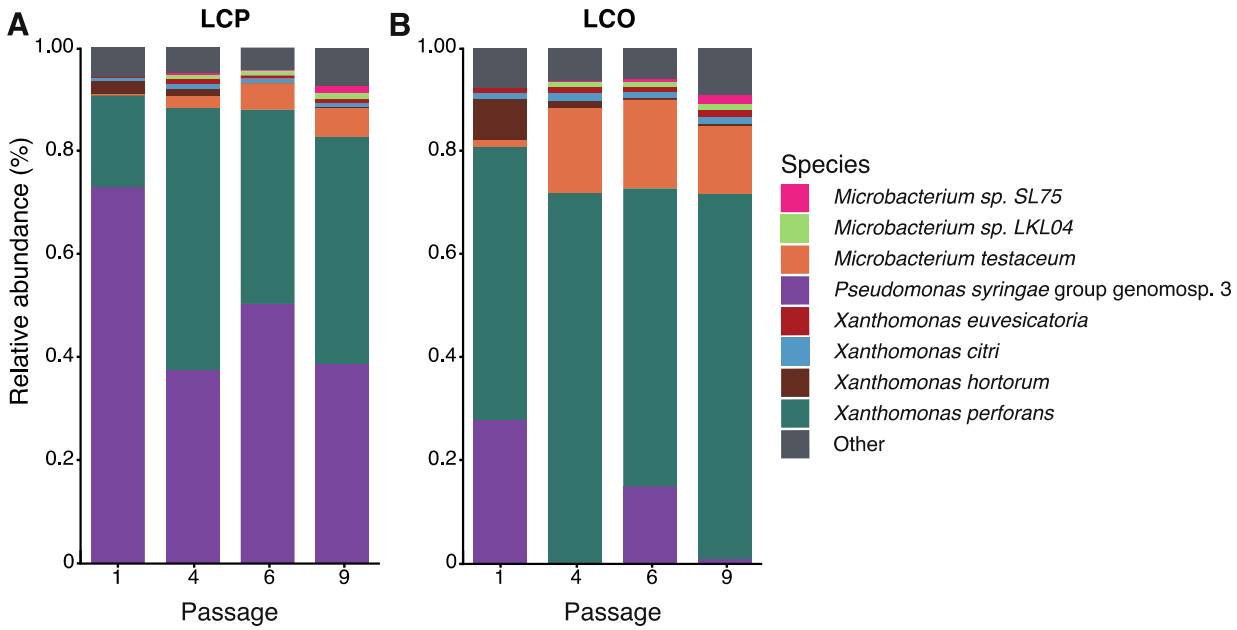
26 **Figure S2.** Fungal composition at the genus level across treatments across passages 1 to 9.

27 The relative abundance (%) presented for genera >3%. 'Other' includes all taxa assigned

28 <3%.

29



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31 **Figure S3.** Species relative abundance for (**A**) Leaf community with *Pto* (LCP) and (**B**) Leaf

32 community only (LCO) microbiomes. Dominant genera assigned and estimated using Kraken

33 2 at passages 1, 4, 6, and 9 representing different stages of disease progression throughout

34 the passaging experiment. 'Other' includes all taxa assigned <3%.

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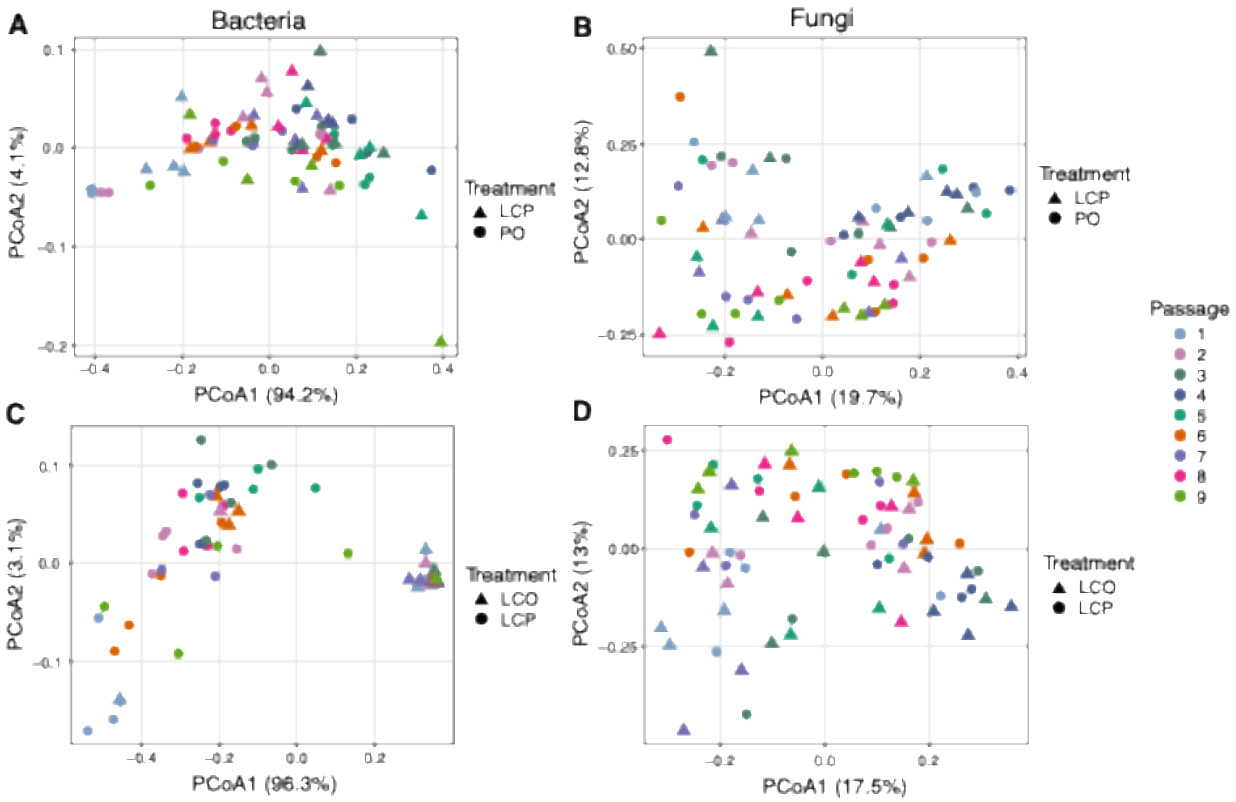

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37 **Figure S4.** Principal coordinates analysis (PCoA) presented the distribution of LCP vs PO

38 treatments for (**A**) bacterial and (**B**) fungal microbiomes, along with LCO vs LCP treatments

39 for (**C**) bacterial and (**D**) fungal microbiomes. * P≤0.05.

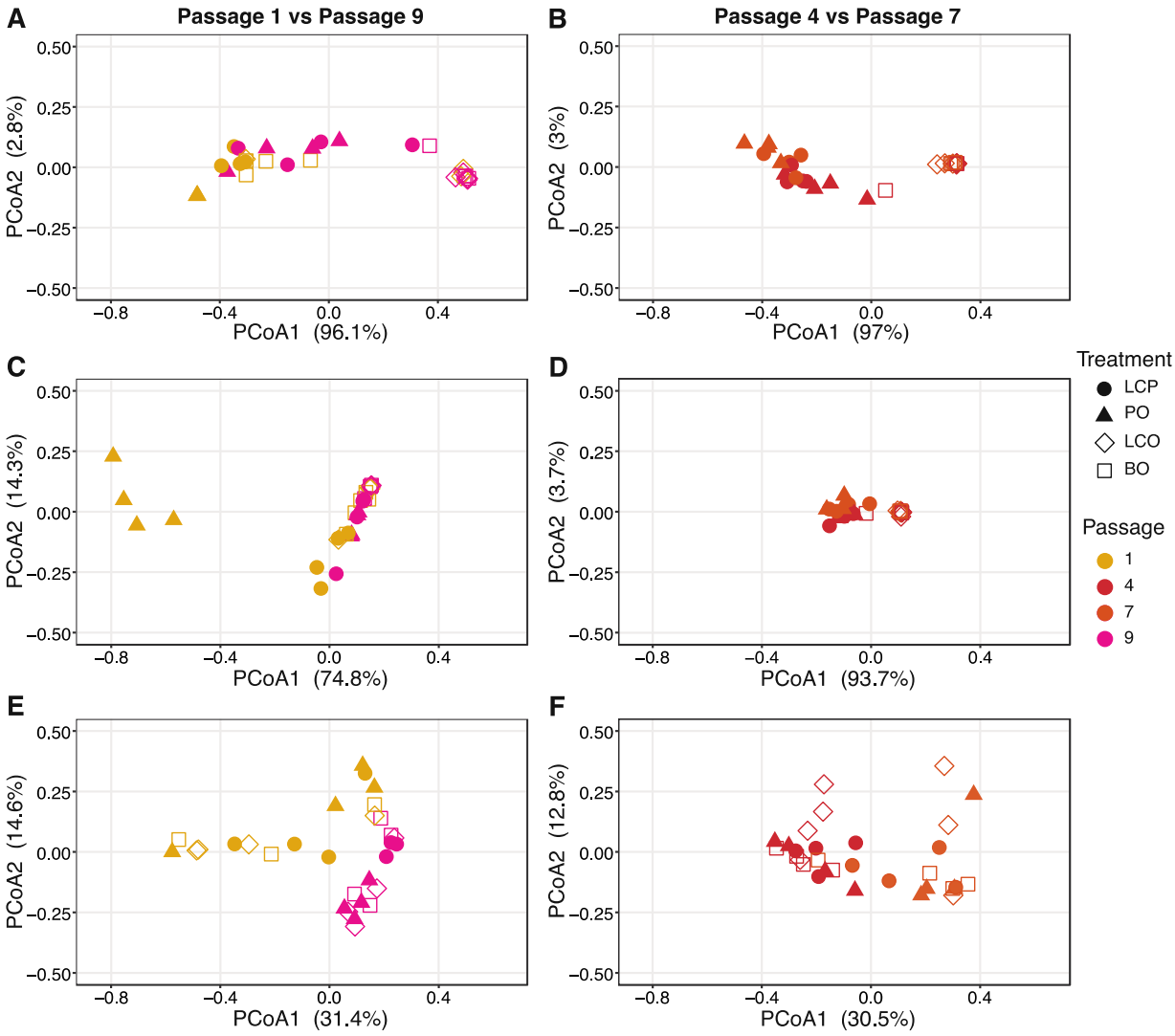

Figure S5. Similarity of microbiomes for bacteria and fungi at key passages related to disease severity. Principal Coordinates Analysis (PCoA) of Bray-Curtis dissimilarity between passages 1 and 9 (low disease before and after disease peak) or passages 4 and 7 (peak and end of peak disease severity) for bacteria (**A** and **B**), bacteria excluding ASV3 identified as *Pseudomonas syringae* (**C** and **D**), and fungi (**E** and **F**).

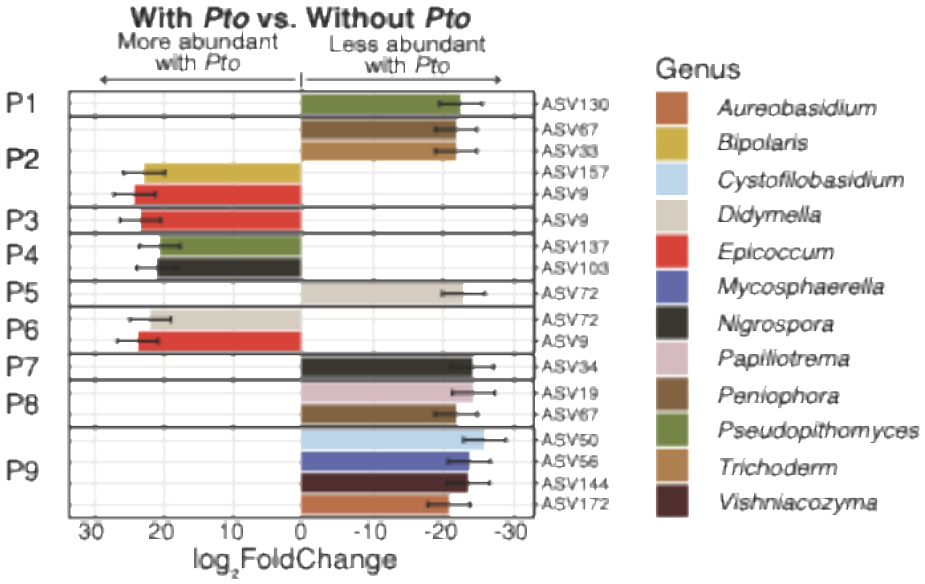

Figure S6. Differentially abundant fungal genera from amplicon sequencing. Significant log_2 fold change of different fungal genera between treatments with *Pto* (LCP and PO) and without *Pto* (LCO and BO) across passages (P≤0.05). Error bars represent standard errors. Bars are coloured by genera.

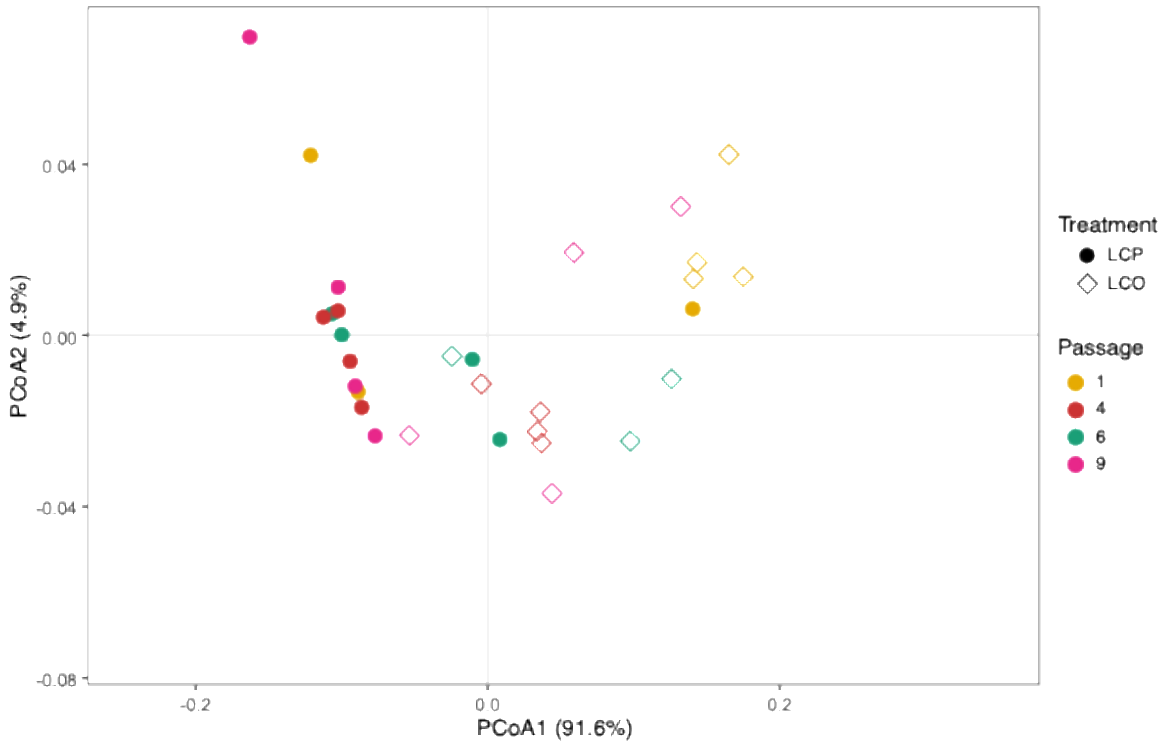

Figure S7. Principal coordinates analysis (PCoA) of Bray-Curtis dissimilarity between LCP and LCO microbiomes from metagenomic sequencing. * P≤0.05.

Table S1. Summary of information from amplicon and metagenomic sequencing

Amplicon		Total reads	Range of reads after quality filtering	Mean number of reads per sample after quality filtering	
Bacteria		3693727	11448 - 38770	21290	
Fungi		3460068	3526 - 35750	9012	
Metagenomic		Mean number of reads	Mean number of reads after quality filtering	Mean number of contigs	Number of PFAM IDs
LCP	P1	3944803	3210461	8246	12969
	P4	4375087	3506557	7842	14272
	P6	4911585	3644367	6845	13306
	P9	4589054	3415975	9203	14802
LCO	P1	4586201	2745397	15550	14992
	P4	4360172	3124946	14198	10779
	P6	4239668	2890412	14198	16458
	P9	4337250	2518476	32507	13165

Table S2. Shannon diversity statistics compared between treatments with *Pto* (LCP and PO) and without *Pto* (LCO and BO) using the Kruskal–Wallis test.

Passage	Bacteria	Fungi
1	0.21375	0.96000
2	0.12857	0.96000
3	0.00144*	0.96000
4	0.00036*	0.96000
5	0.00036*	0.96000
6	0.65000	0.96000
7	0.00036*	0.96000
8	0.00036*	0.96000
9	0.00056*	0.96000

[a] P-value FDR adjusted across all passages

* Indicates statistical significance (P≤0.05)

Table S3. PERMANOVA on Bray-Curtis dissimilarities of bacterial and fungal microbiomes between selected passages and treatments in supplemental.

Comparison	Microbiome	Factor	R^2	P value
Passages 1 vs 9	Bacteria	Passage	0.24240	0.001*
		Treatment	0.50968	0.001*
		Passage:Treatment	0.06772	0.052
	Bacteria excluding AVS3	Passage	0.18811	0.001*
		Treatment	0.07277	0.712
		Passage:Treatment	0.09862	0.284
	Fungi	Passage	0.18811	0.001*
		Treatment	0.07277	0.707
		Passage:Treatment	0.09862	0.275
Passages 4 vs 7	Bacteria	Passage	0.23896	0.005*
		Treatment	0.89337	0.001*
		Passage:Treatment	0.03425	0.010*
	Bacteria excluding AVS3	Passage	0.01566	0.081
		Treatment	0.84129	0.001*
		Passage:Treatment	0.01949	0.315
	Fungi	Passage	0.10841	0.001*
		Treatment	0.09800	0.141
		Passage:Treatment	0.05875	0.801
LCP vs PO	Bacteria	Treatment	0.05239	0.004*
	Fungi	Treatment	0.00901	0.762
LCO vs LCO	Bacteria	Treatment	0.66285	0.001*
	Fungi	Treatment	0.01282	0.311

[a] P-value FDR adjusted across all passages

* Indicates statistical significance (P≤0.05)

Table S4. Pfam IDs selected for each functional category from Eggnogmapper2.

Functional category	Pfam ID
Ammonia	Amidase AstB
Bacteriocins	Colicin_M Colicin_Pyocin Colicin_V CreD Pyocin_S PyocinActivator
Bacterioferritin	Ferritin
Chitinase I	Glyco_hydro_19
Formamide	Arginase
Enterobactin	4HBT
Non-ribosomal peptide synthase (NRPS)	AMP-binding,AMP-binding_C,Condensation,PP-binding, Thioesterase
Phage	Collar DUF3693 Gp49 HDPD Lambda_tail_I P2_Phage_GpR Phage_AlpA Phage_base_V Phage_cap Phage_capsid Phage_connect_1 Phage_CP76 Phage_fiber_2 Phage_GPD Phage_GPL Phage_GPO Phage_holin Phage_H_T_join Phage_P2_GpU Phage_portal Phage_sheath_1,Phage_sheath_1C Phage_TAC_7 Phage_tail Phage_term_smal Phage_TTP_11 Phage_tube PhageMin_Tail NfrA_C Tail_P2_I Tail_tube Tape_meas_lam_C
Phenazine	PhzC-PhzF
Polyketide synthases	Ketoacyl-synt Ketoacyl-synt_2
Proteases	ClpB_D2-small CLP_protease Clp_N Peptidase_C1_2 gag-asp_proteas PA Peptidase_S8 PrsW-protease TerD,Trypsin_2
Siderophore production	FhuF,IucA_IucC SIP TonB_dep_Rec ABC_membrane,ABC_tran
Terpene	Terpene_synth
Terpenoids	Prenyltransf

Type II secretion system	T2SS T2SSC T2SSE T2SSF T2SSJ	T2SSL T2SSK T2SSM T2SSN
Type IV secretion system	T4SS T4BSS_DotI_IcmL T4BSS_DotH_IcmK	
Type VI secretion system	T6SS_TssF T6SS_TssG T6SS_VasE T6SS_VipA T6SS-SciN	
Type VI secretion effectors	T6SS_HCP	
Type VII secretion system	T7SS_ESX1_EccB	

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