Supplemental Information for:

Investigation into how *Odontotermes obesus* maintains a predominantly *Termitomyces* monoculture in their fungus combs suggests a potential partnership with both fungi and bacteria

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Supplementary Note 1: Sample Collection/Mounds used:

Different castes of *O. obesus* were collected from two different mounds of the IISER Mohali campus. Fresh fungus combs were collected across three months (August-October, 2018) from two mounds to set up the incubation to decay for 120 hrs. The collected combs were immediately kept in sterile plastic containers and were then crushed with autoclaved pestles, divided into roughly 0.5 g portions, and incubated at 30 °C inside sterilized glass containers. For each day, three such containers were set up from each mound. Major workers, minor workers and nymphs collected from the combs were kept in separate sterile vials. Regular visits were made to these mounds, in anticipation of emerging alates, after the first monsoon shower in June 2018. Male and female alates were collected directly from the mounds and kept individually in sterile vials. DNA was extracted from individual termite and comb samples using the CTAB (Cetyl Trimethyl Ammonium Bromide) method.

Supplementary Note 2: DNA extraction

1 ml of CTAB buffer containing 100 mM Tris (pH 8), 2% CTAB, 1.4 M NaCl, 20 mM EDTA, 1% β-mercaptoethanol and 0.3 g mL^{-1} proteinase K was used for DNA extraction from termite castes and fungus comb samples. The samples were crushed in the buffer and then incubated at 60˚C for 30 minutes. These were then subjected to Phenol: Chloroform: Isoamyl alcohol (PCI: 25:24:1) precipitation and the purified DNA pellet obtained was dissolved in 1X TE (pH 8) buffer. Extracted DNA from fungus combs often have extensive humic acid contamination which can hinder downstream PCR reactions. To remove this humic acid, the precipitation step with Phenol: Chloroform: Isoamyl alcohol was repeated until the final pellet obtained was white in color. DNA samples were used for PCR only if the 260/280 nm ratio were within the range of 1.3-1.9, which were obtained through a NanoDrop Spectrophotometer 2000 (Thermo-Fisher Scientific). The 260/280 nm ratio was variable for the DNA extracted from the comb samples due to the natural presence of humic acid[1]. DNA from the combs was extracted consecutively for six days, i.e., 0 hrs to 120 hrs. For 0 hrs, DNA was extracted from each portion separately within 3 hours of the collection.

Supplementary Note 3: Identification of isolated fungal strains

DNA from morphologically distinct fungi were extracted with CTAB buffer and amplified with the primer set ITS4/ITS5[2]. The amplification reaction was prepared to a final volume of 20 µl containing: 14.5 µl sterile distilled water, 0.4 µl dNTPs (10 µM), 2 µl 17.5 mM buffer, 0.5 µl of each primer (10 µM), 2 µl template and 0.1 µl *Taq* Polymerase (Himedia). PCR was performed under following conditions: an initial denaturation step at 95°C for 3 minutes, followed by 37 cycles of denaturation (95°C, 45 seconds), annealing (56°C, 45 seconds), extension (72°C, 1 minute) with a final extension at 72°C for 10 minutes.

The PCR products were cleaned with Exonuclease I and Shrimp alkaline phosphatase and then sequenced with BigDye® Terminator v.3.1 cycle sequencing kit for both strands. The chromatograms obtained were cleaned with Sequencher v.5.2.4 (GeneCodes Corp.) and the sequences were manually edited with Bioedit v.7.0.5.3[3]. Taxonomic identification of these sequences was achieved through BLAST with the parameters of >90% sequence identity and/or the first hit. The sequences were aligned with other similar homologues, obtained from NCBI, using the ClustalW in Bioedit. The Maximum likelihood phylogenetic tree of aligned sequences was constructed using MEGAX v.10.1.7[4] with 1000 bootstrap replicates. Suitable substitution models for various phylogenetic analyses were also obtained through MEGAX[4]. All the fungi selected for use in these experiments had unique sequence profiles in their Internal Transcribed Spacer (ITS) gene.

Supplementary Note 4: Nanopore Sequencing

- **Sample preparation and PCR amplification**

Two separate sets of nanopore runs were done. The first was to identify the pan-mycobiota and the second was to identify the microbiota of the degrading combs. To obtain a comprehensive assessment of fungal diversity, three separate DNA extractions per mounds were pooled in equimolar concentrations. This was done for all the termite castes, except alates, where three individual DNA extractions from males and females were pooled (Table S2 and S3). Five different DNA samples (major worker, minor worker, nymph, male alate and female alate) were thus prepared for mycobiota estimation and the other six comb samples (0 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs) were prepared for both micro- and mycobiota estimation on the Nanopore platform.

To identify the mycobiota present within the termite castes and fungus comb, the ITS fragment was amplified using the primers ITS5/ITS4[2] whereas to characterize the microbiota in a degrading fungus comb, *16S* rRNA[5] gene regions was amplified. The amplification reaction was prepared to a final volume of 20 µl containing: 14.5 µl sterile distilled water, 0.4 µl dNTPs (10 µM), 2 µl 17.5 mM buffer, 0.5 µl of each primer (10 µM), 2 µl template and 0.1 µl *Taq* Polymerase (Himedia). PCR conditions involved an initial denaturation step at 95°C for 3 minutes, followed by 33 cycles of denaturation (95°C, 45 seconds), annealing (56°C, 45 seconds), extension (72°C, 1 minute) with a final extension at 72°C for 10 minutes. PCR products were cleaned using Wizard® SV Gel and PCR clean-Up System (Promega). Purified PCR products were quantified using NanoDrop 2000 (Thermo Scientific) and Qubit 3.0 Fluorometer (Life Technologies).

- **Library prepartion**

An initial library preparation was done using the SQK-LSK108 Ligation Sequencing Kit 1D-vR9 (Oxford Nanopore Technologies) by Ultra II End-prep enzyme mix. The resultant mixture was purified using 1X AMPure XP beads and eluted in nuclease-free water. These were individually ligated with barcode adaptors and purified using 1X AMPure XP beads and eluted in nuclease-free water. These different samples were then individually barcoded with unique barcodes using the EXP-PBC001 PCR barcoding kit I (https://community.nanoporetech.com/protocols).

Barcoded samples were then purified using Wizard® SV Gel and the PCR clean-Up System (Promega) and quantified using Qubit 3.0 Fluorometer (Life Technologies). All the twelve barcoded samples were

pooled in equal amount (20-25 ng/µl) before being loaded on the Nanopore flowcell. 5 µl of λ phage DNA was added to the library to serve as a control. Samples were sequenced on a MinION platform (MinION Mk1B). The sequencing library was run on FLO-MIN106 flowcell for 48 hours using MinKNOW software with the protocol *NC_48Hr_sequencing_FLO-MIN106_SQK-LSK108_plus_Basecaller*.

- **Data analysis workflow**

After the completion of the run, sequences were first separated according to their barcodes by using the program ont-albacore (Oxford Nanopore Technologies Ltd.) These were then converted to FASTA and FASTQ files using *Poretools* (v 0.5.1) for downstream analysis[6]. High-quality reads (average read quality score ≥ 10) were selected using the program *Nanofilt*[7]. The obtained high-quality sequences were then further processed where the barcodes and adaptors were removed using Porechop (https://github.com/rrwick/Porechop). The sequencing exhibited the average accuracy of about 89% which was estimated by aligning control λ phage DNA with existing NCBI λ sequences (NC_001416.1) using LAST v. 973. The fungal OTU's were identified by comparing them against the fungus repository from NCBI FTP site and the bacterial sequences generated from the six comb samples (0 hrs- 120 hrs) were identified by comparing them against the customized microbial repository made by combining 16S rRNA gene fragments from NCBI FTP site and the DictDb v 3.0 database[8] . Annotation was done using LAST v 973, with the following parameters: match score of 1, gap opening penalty of 1, and gap extension penalty of 1[9]. The identified reads were sorted from the phylum to the genus level and their relative abundances were calculated.

Supplementary Note 5: *In vitro* anti-fungal assays with *Pseudoxylaria* and *Termitomyces*

In vitro interaction experiments were done to identify whether *Termitomyces* and *Pseudoxylaria* can prevent the growth of the fungal contaminants. A simple explanation for the inhibitory capabilities seen for both *Pseudoxylaria* and *Termitomyces* could be the depletion of nutrients in the PDA plates. However, control experiments using previously used media to grow these fungi indicate the presence of sufficient nutrients in these plates for adequate fungal growth (Fig. S14).

Fig. S1: Setting up the fungus combs to decay

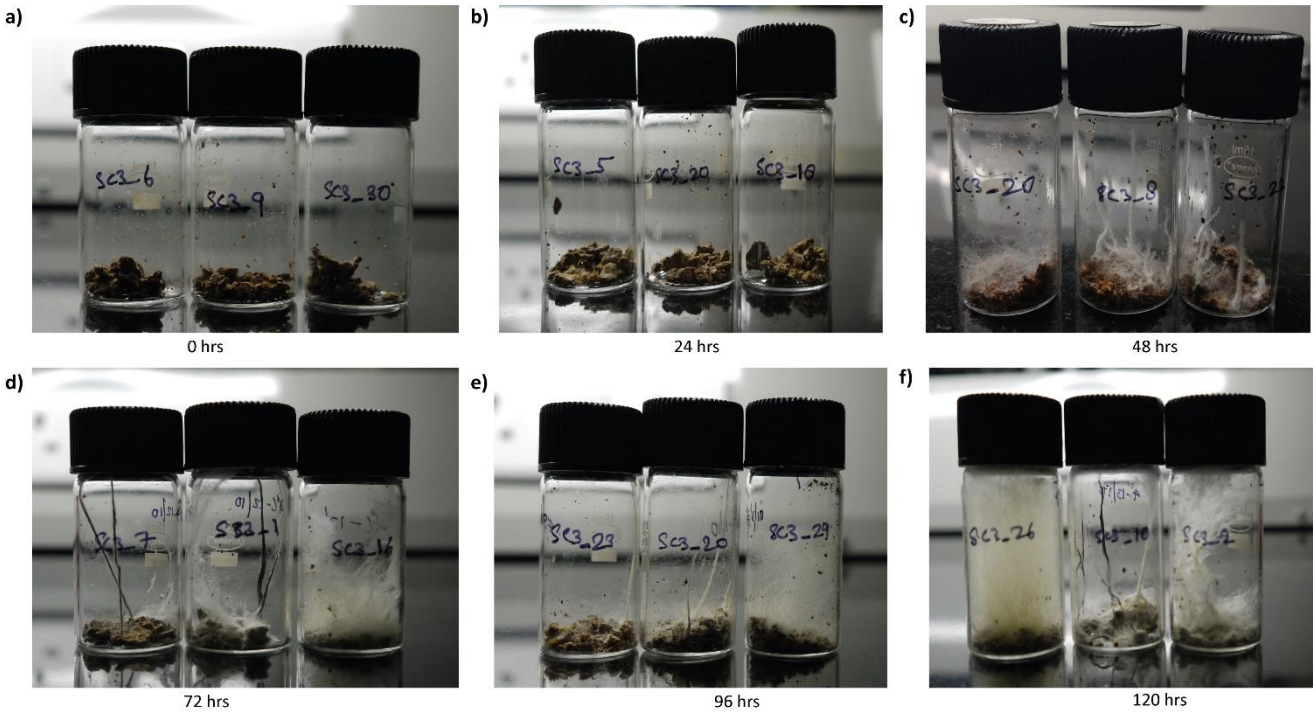


Fig. S1: Crushed fungus combs inside sterilized glass containers. Fresh fungus combs were collected across three months (August-October, 2018) from two different mounds and were crushed with autoclaved pestles, divided into roughly 0.5 g portions. For each day, three such containers were set up from each mound which were further used for DNA extraction and sequencing.

Fig. S2: A Phylogenetic tree of *Pseudoxylaria* using α-actin gene

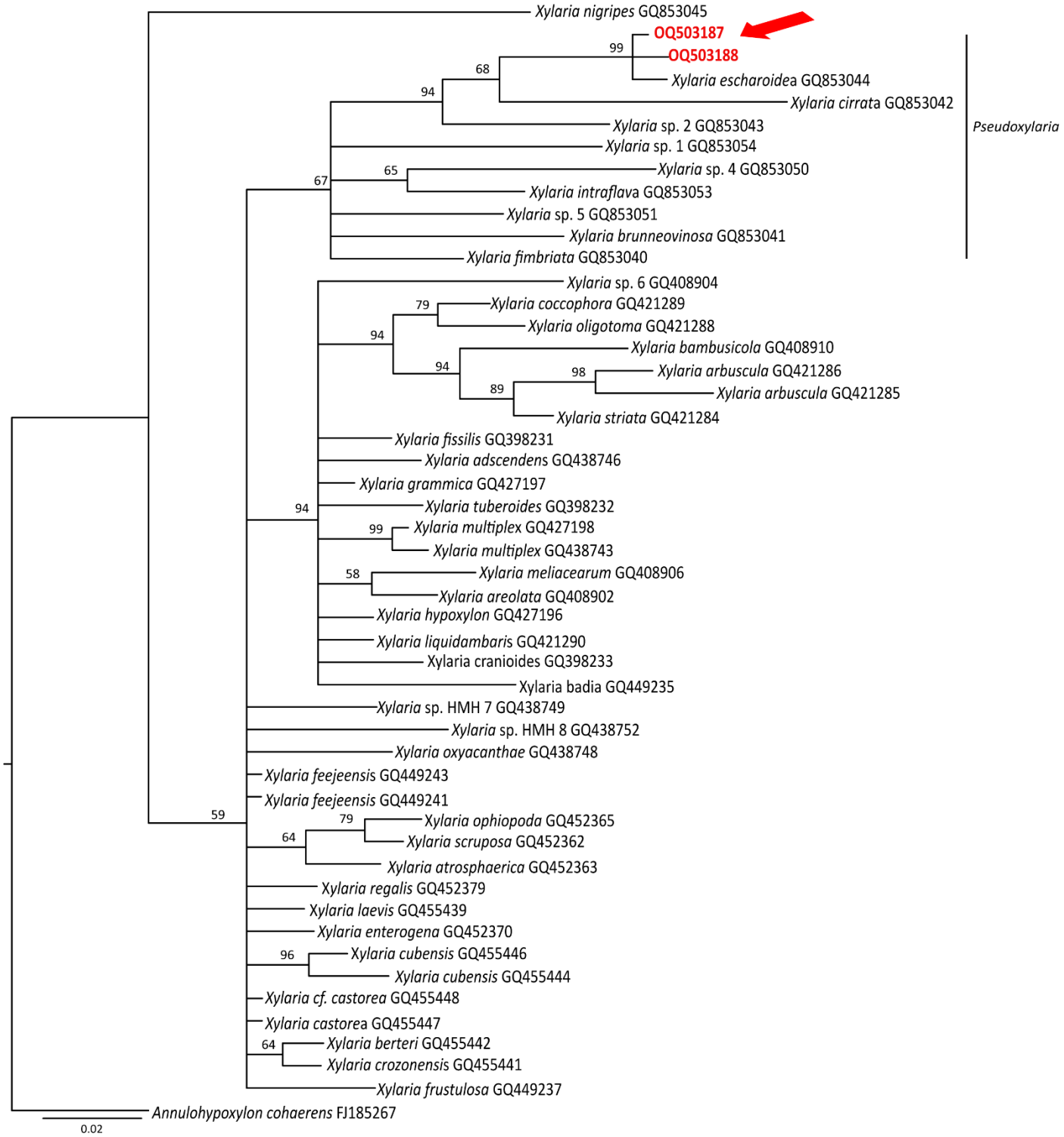
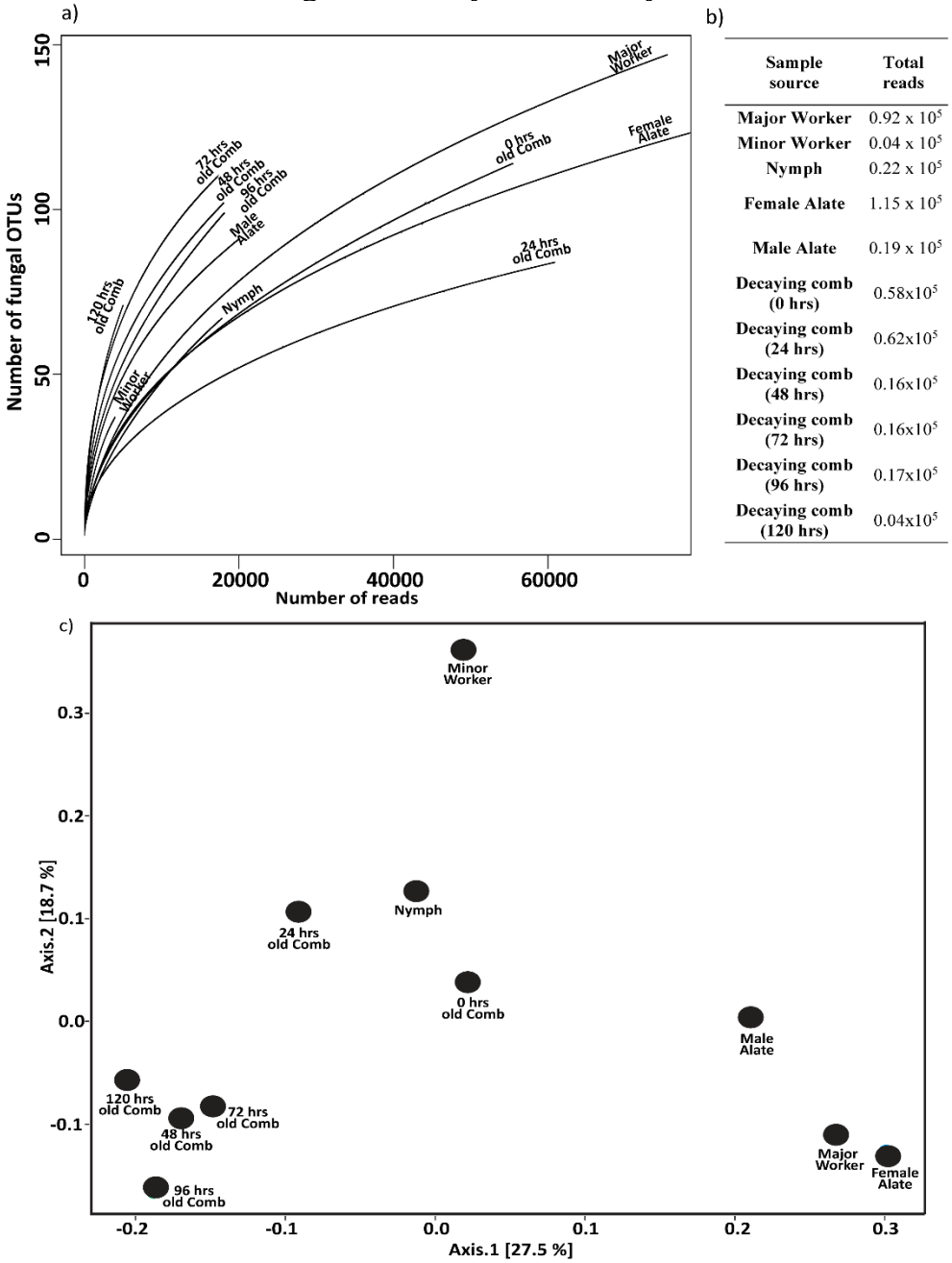


Fig. S2: A phylogenetic tree of *Pseudoxylaria* generated by using Bayesian analysis from the α-actin gene. *Annulohypoxylon cohaerens* was used as the outgroup taxa. Taxa in bold red and also indicated with a red arrow were obtained from this study and other all taxa were obtained from Hsieh et al.[10].

Fig. S3: A Phylogenetic tree of *Pseudoxylaria* using ITS gene

Fig. S3: A phylogenetic tree of *Pseudoxylaria* generated by using Bayesian analysis from the ITS gene. *Annulohypoxylon cohaerens* was used as the outgroup taxa. Taxa in bold red and also indicated in red arrow were obtained in culture and other all taxa were obtained from the Nanopore sequencing of this study.

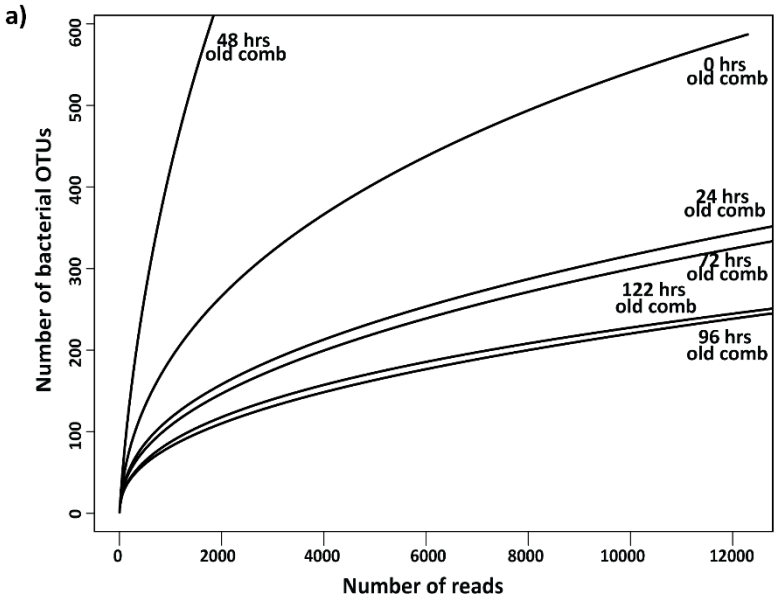
Fig. S4: Pan-mycobiota analysis



Sample source	Total reads
Major Worker	0.92×10^5
Minor Worker	0.04×10^5
Nymph	0.22×10^5
Female Alate	1.15×10^5
Male Alate	0.19×10^5
Decaying comb (0 hrs)	0.58×10^5
Decaying comb (24 hrs)	0.62×10^5
Decaying comb (48 hrs)	0.16×10^5
Decaying comb (72 hrs)	0.16×10^5
Decaying comb (96 hrs)	0.17×10^5
Decaying comb (120 hrs)	0.04×10^5

Fig. S4: The pan-mycobiota analysis of degrading fungus comb a) Rarefaction analysis of fungal OTUs from six different sources of *O. obesus* colony, b) Number of reads obtained to identify the mycobiota of *O. obesus* from Nanopore Sequencing c) PCoA similarity analysis of six samples of this study using weighted Unifrac distance.

Fig. S5: Microbiota of the decaying combs



b)

Sample source	Total reads
Decaying comb (0 hrs)	0.14×10^5
Decaying comb (24 hrs)	0.94×10^5
Decaying comb (48 hrs)	0.38×10^5
Decaying comb (72 hrs)	1.54×10^5
Decaying comb (96 hrs)	0.63×10^5
Decaying comb (120 hrs)	1.33×10^5

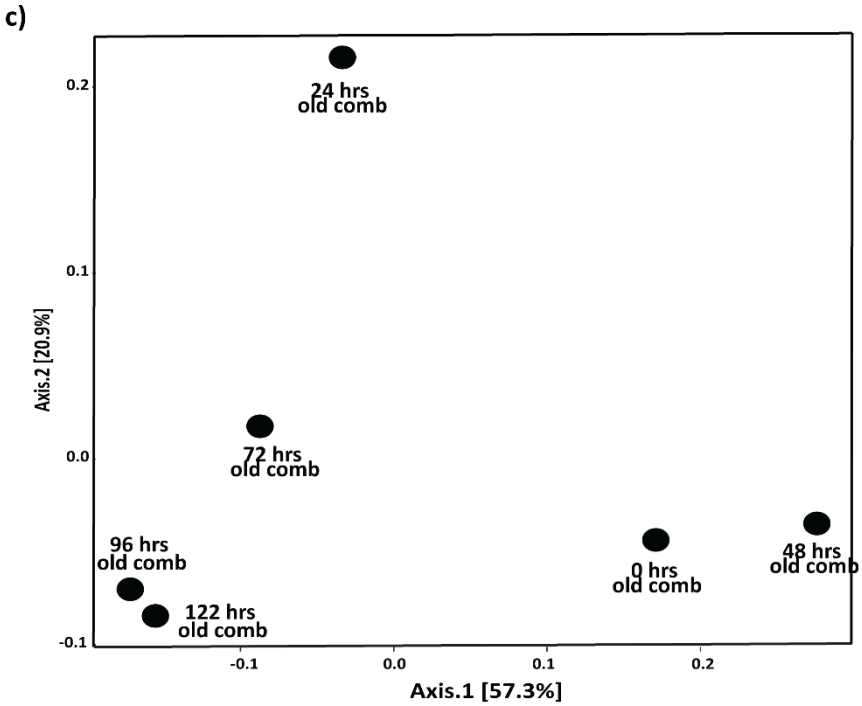


Fig. S5: Microbiota analysis of degrading fungus comb. a) Rarefaction analysis of the six samples, b) Number of reads obtained to identify the microbiota of *O. obesus* from Nanopore sequencing c) Weighted Unifrac PCoA analysis.

Fig. S6: Relative abudances of the bacterial genera in the decaying combs

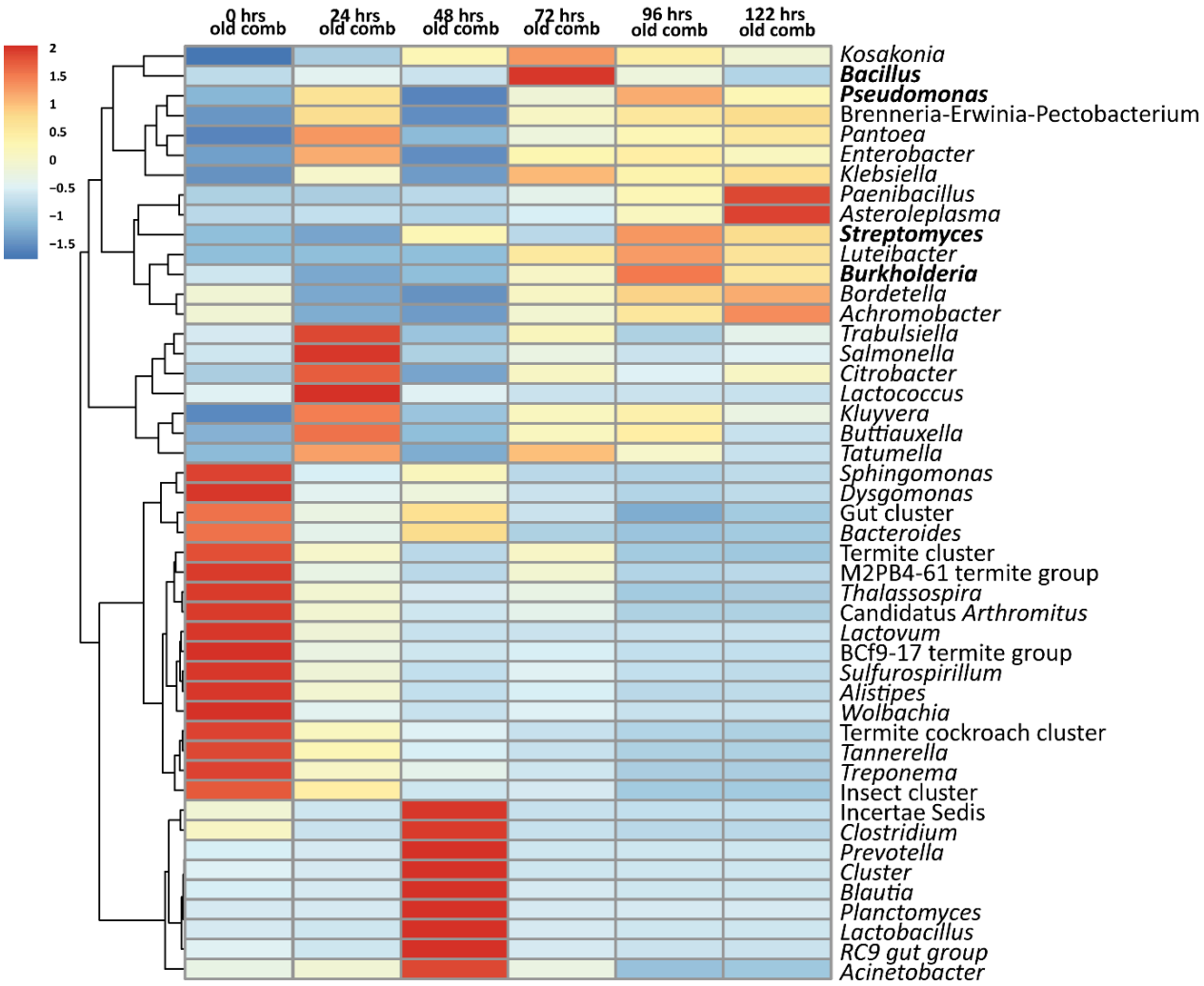


Fig S6: Heatmap of the bacterial genera with relative abundance of at least 0.05% in any of the samples.

Fig. S7: Change in abundances of *Termitomyces*, *Pseudoxylaria* and bacterial mutualists in decomposing fungus comb.

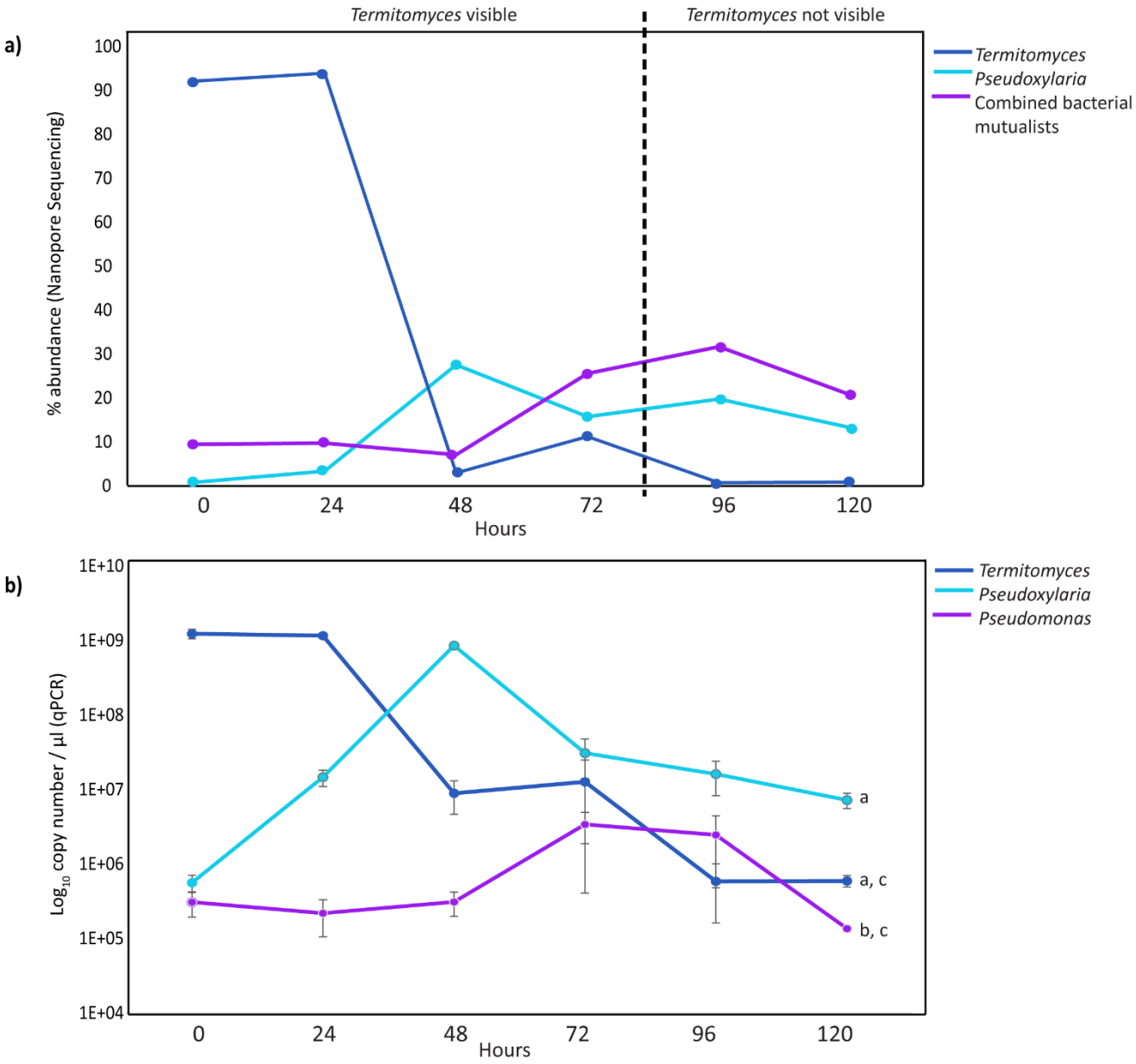


Fig. S7: a) Change in absolute abundances of *Termitomyces*, *Pseudoxylaria* and bacterial mutualists in different decomposing stages of fungus comb. b) Change in copy numbers of *Termitomyces*, *Pseudoxylaria* and *Pseudomonas* as determined by qPCR analysis. One- way Anova showed significant variation between the groups (p= 0.00081, F= 7.512, df= 2). Post hoc Tukey tests revealed insignificant pairwise site differences in the copy numbers of *Termitomyces* vs *Pseuodxylaria* (p= 0.102) and *Pseudoxylaria* and *Pseudomonas* (p= 0.364).

Fig. S8: Change in abundances of *Termitomyces*, *Pseudoxylaria* and other fungi in decomposing fungus comb.

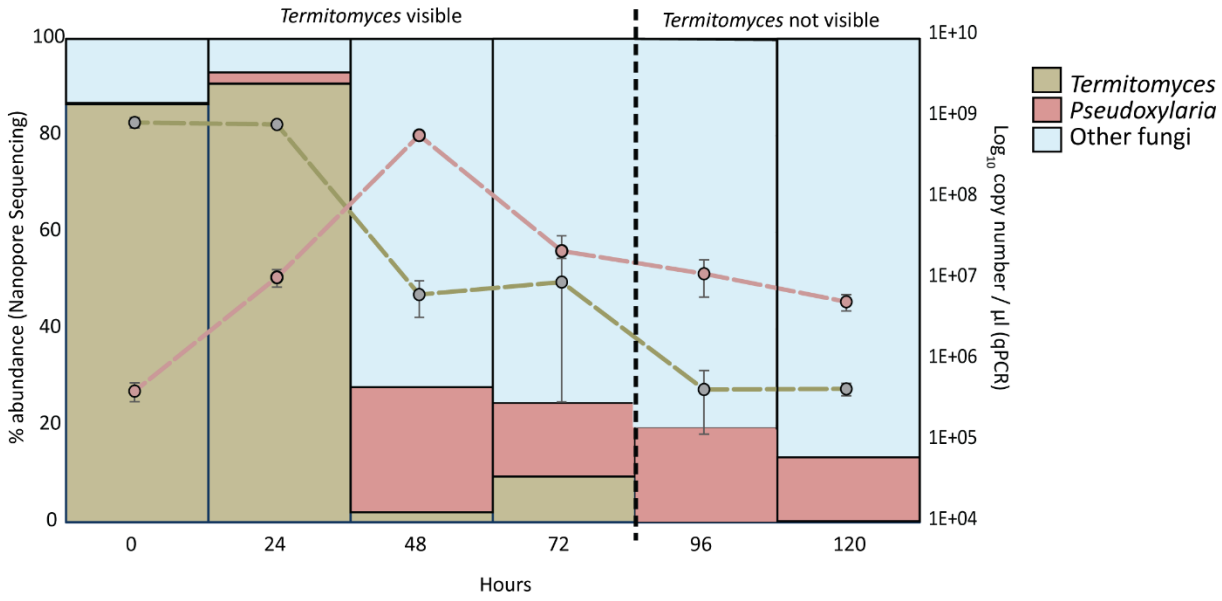


Fig. S8: Change in abundance (in percentages) of *Termitomyces*, *Pseudoxylaria* and other fungi, and $\log_{10}$ copy numbers of *Termitomyces* and *Pseudoxylaria* in different stages of the decay of the fungus comb as determined by Nanopore Sequencing (bar graph) and qPCR (line graph).

Fig. S9: Interaction assay between *Pseudoxylaria* and *Termitomyces*

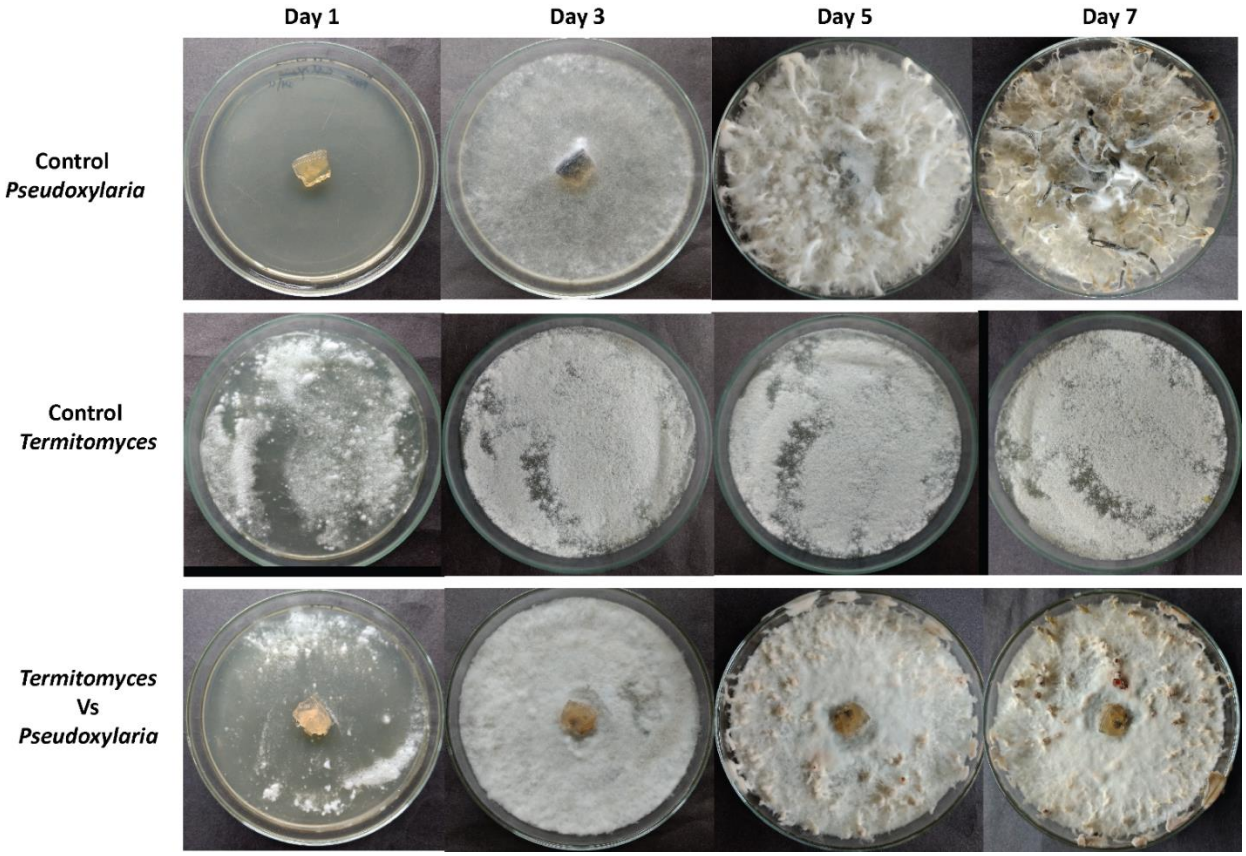


Fig. S9: Interaction assay between *Pseudoxylaria* and *Termitomyces*. Interaction is represented with its control and test plate. *Pseudoxylaria* easily grow over *Termitomyces* indicating that the crop fungus cannot prevent the most prominent weedy fungus. Also with its faster growth rate, *Pseudoxylaria* can be a more efficient inhibitor of non-specific fungi than *Termitomyces*.

Fig. S10: Growth of non-specific fungus on differently grown/incubated *Termitomyces*

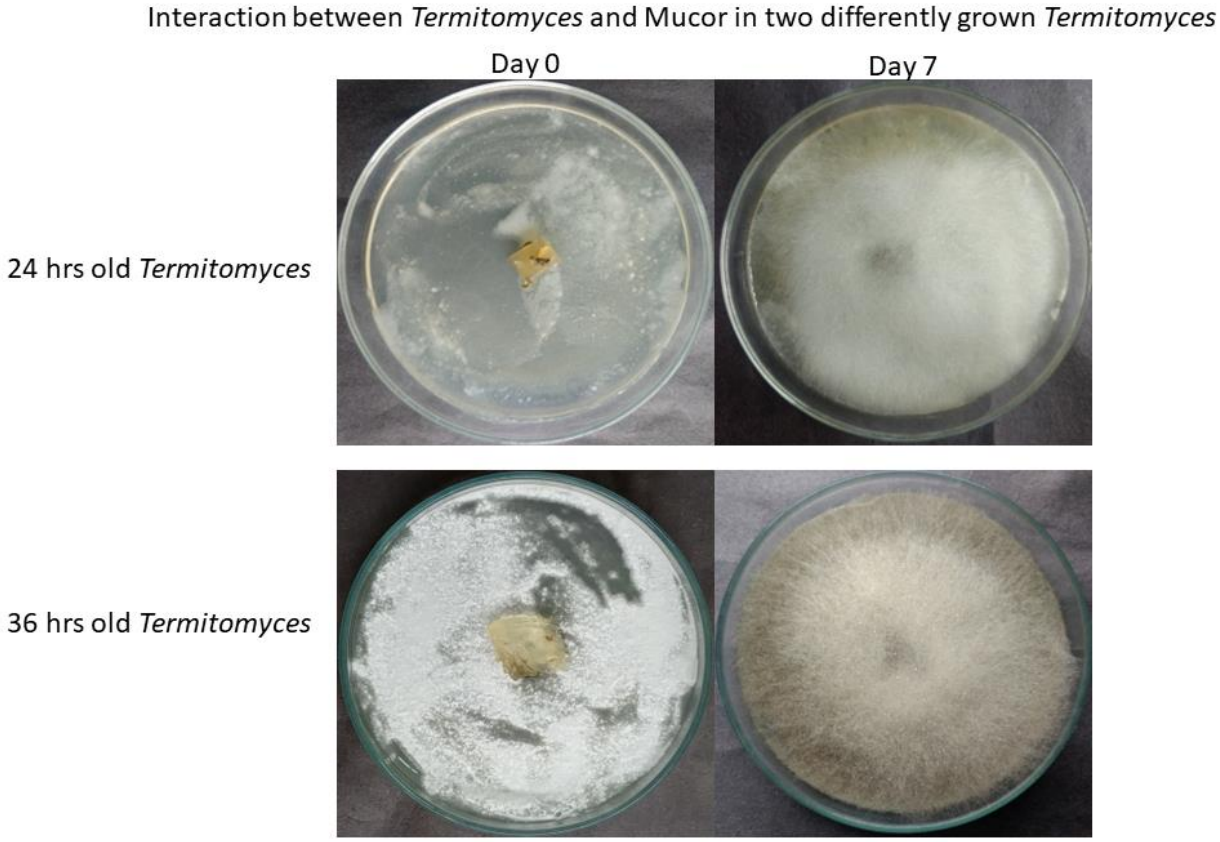


Fig. 10: Comparison of growth of *Mucor* on two differently grown plates of *Termitomyces*. No difference in the interaction was found even in the presence of well grown Termitomyces

Fig S11: Comparison of growth rate of *Pseduoxylaria* and *Termitomyces*

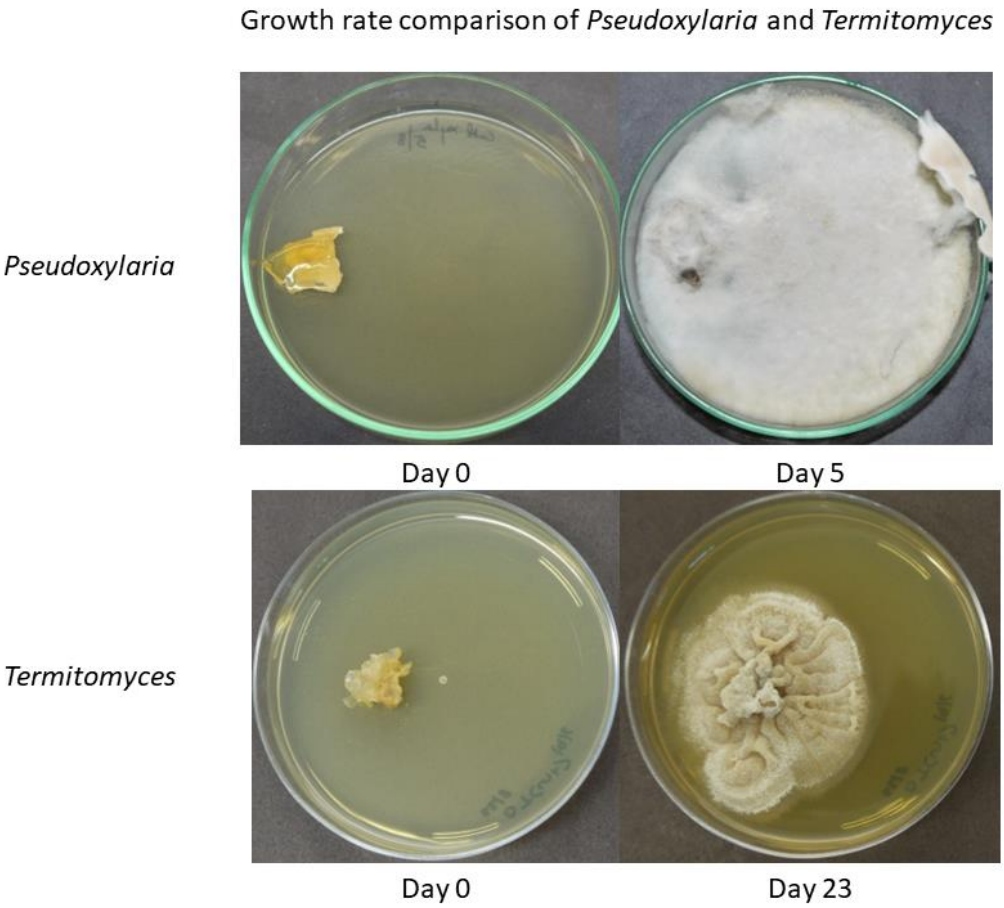


Fig. S11: Pure cultures of *Pseudoxylaria* and *Termitomyces* to see the differences in their growth rate. *Pseudoxylaria* being a fast growing fungus takes around five days to completely cover a petriplate whereas on the other hand *Termitomyces* takes around 30 days to fill half of the petriplate.

Fig. S12: *Termitomyces* Vs Fungal contaminants

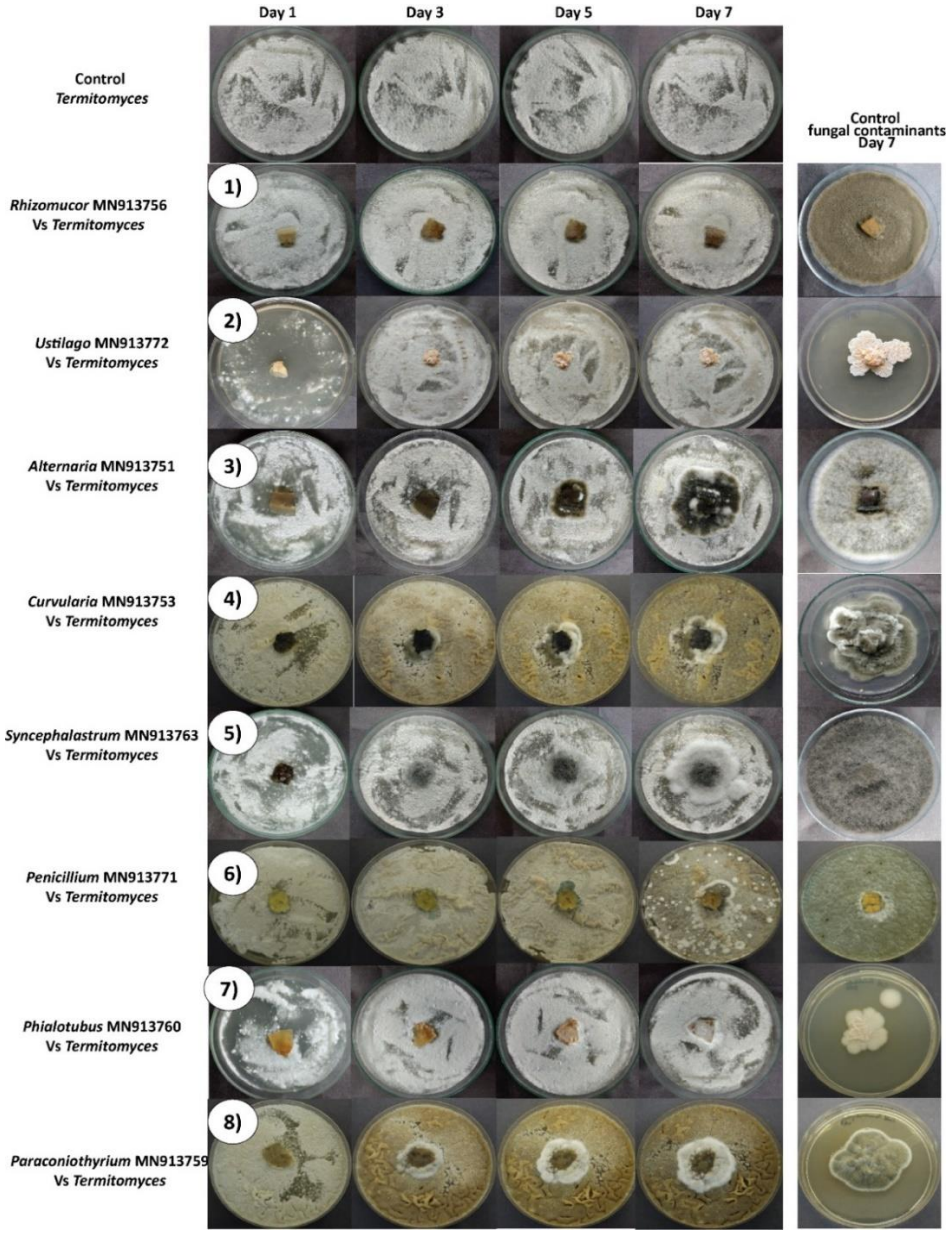


Fig. S12: Interaction assay of *Termitomyces* against fungal contaminants. All the interactions are categorized into three types: **1)-8) more than 50% inhibition 8) - 14) less than 50 % inhibition** and **15)-16) negigible inhibition** by the *Termitomyces*. Each interaction is represented with its fungal and *Termitomyces* controls and a representative test plate. *Termitomyces* showed maximum inhibition (more than 50%) against *Alternaria*-MN913751, *Curvularia*-MN913753, *Rhizomucor*-MN913756, *Syncephalastrum*-MN913763 and *Ustilago*-MN913772. The other nine fungi, *Aspergillus*-MN913749, *Aspergillus*-MN913750, *Diaporthe*-MN913762, *Fusarium*-MN913754, *Lasiodiplodia*-MN913758, *Paraconiothyrium*-MN913759, *Penicillium*-MN913771, *Phialotubus*- MN913760 and *Phoma*-MN913761 showed less than 50% inhibition. However, *Mucor*-MN913757 and *Trichoderma*-MN913767 overgrew *Termitomyces*, showing no sign of inhibition. All the photos are from day 7 of the beginning of the experiment.

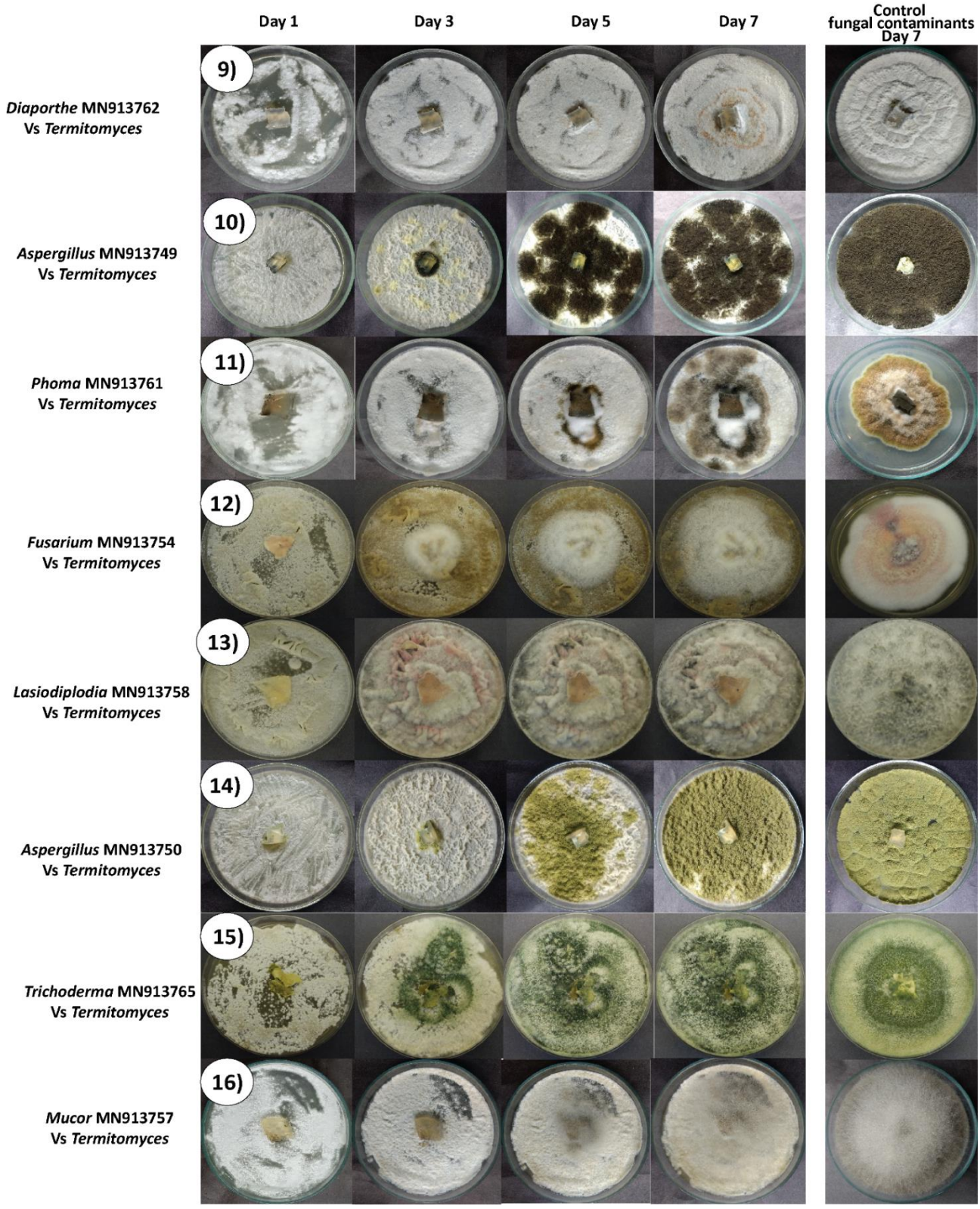


Fig. S12 continued

Fig. S13: *Pseudoxylaria* Vs Fungal contaminants

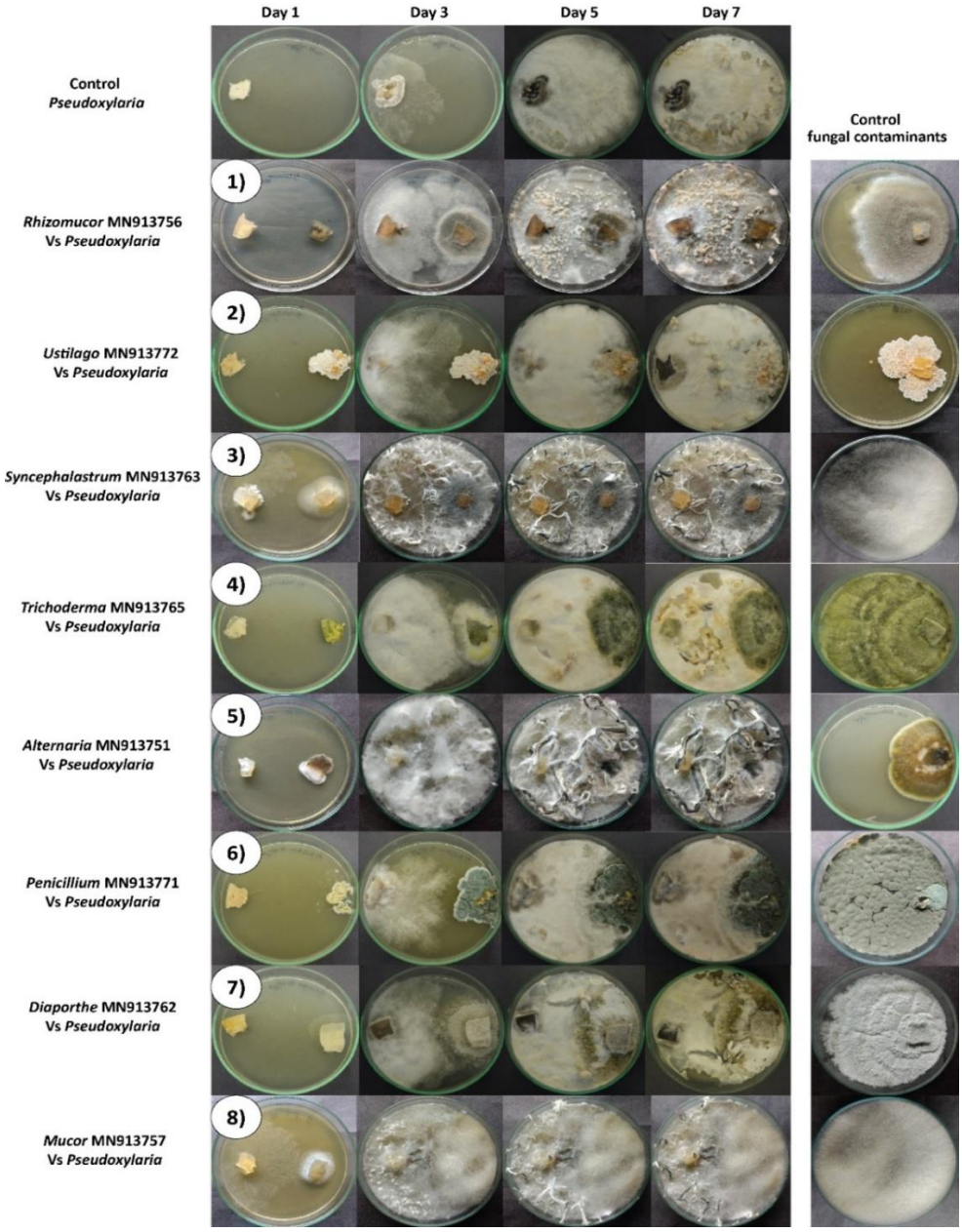


Fig. S13: Interaction assays showing the prevention of the fungal contaminants by *Pseudoxylaria*. All the interactions are categorized into two types: **1)-7) more than 50% inhibition, 8) - 16) less than 50% inhibition**. Each interaction is represented with its fungal and *Pseudoxylaria* control and one representative test plate. More than 50%of inhibition was shown against *Alternaria*-MN913751, *Diaporthe*-MN913762, *Penicillium*-MN913771, *Rhizomucor*-MN913756, *Syncephalastrum*-MN913763, *Trichoderma*-MN913767, and *Ustilago*-MN913772. The other nine fungi (*Aspergillus*-MN91375049, *Aspergillus*-MN913750, *Curvularia*-MN913753, *Fusarium*-MN913754, *Lasiodiplodia*-MN913758, *Mucor*-MN913757, *Paraconiothyrium*-MN913759, *Phialotubus*-MN913760 and *Phoma*-MN913761) were also inhibited by *Pseudoxylaria* but to a lesser degree (less than 50%). All the photos are from day 7 of the beginning of the experiment.

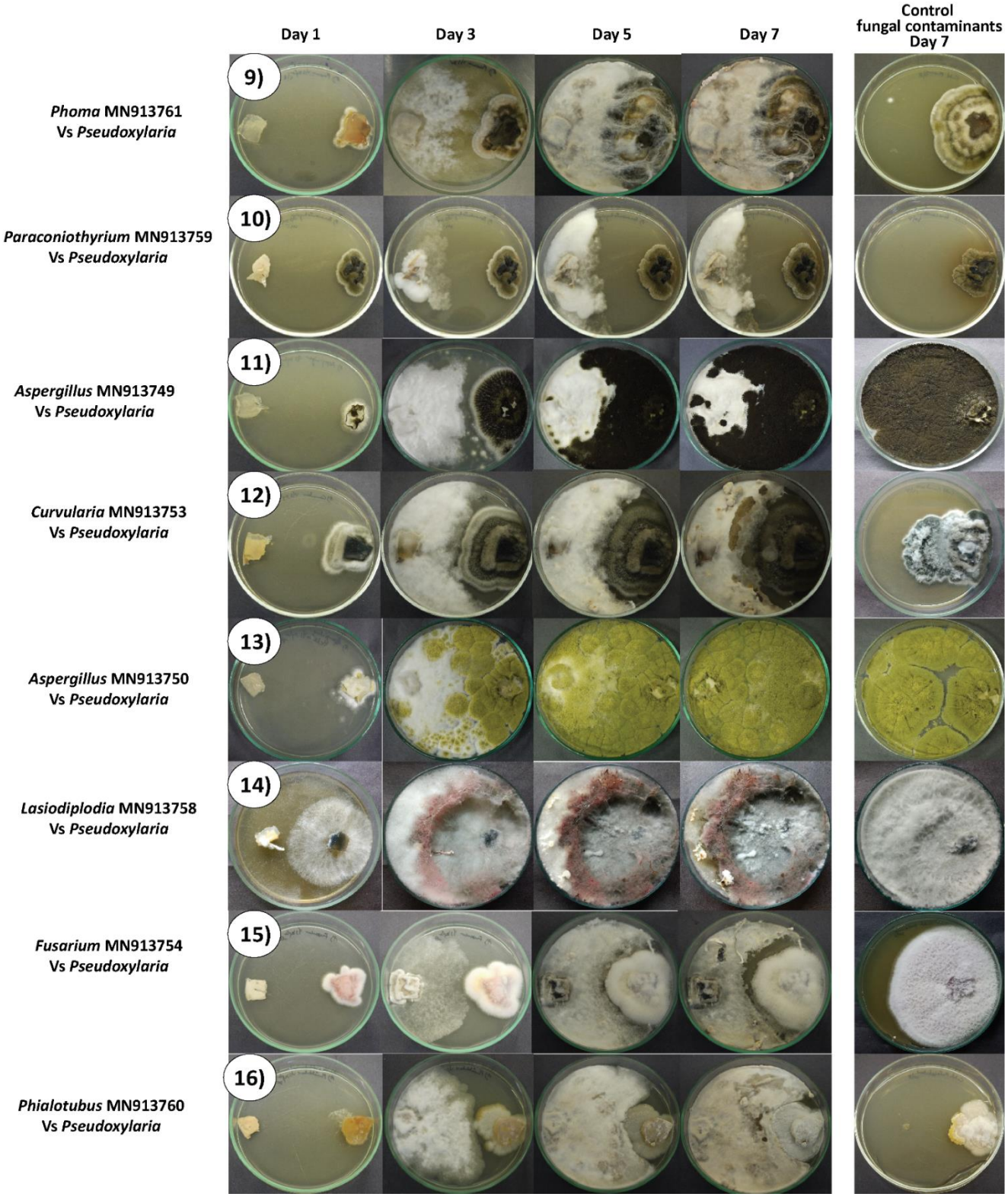


Fig. S13 continued.

Fig. S14: Re-using media from the test experimental plates

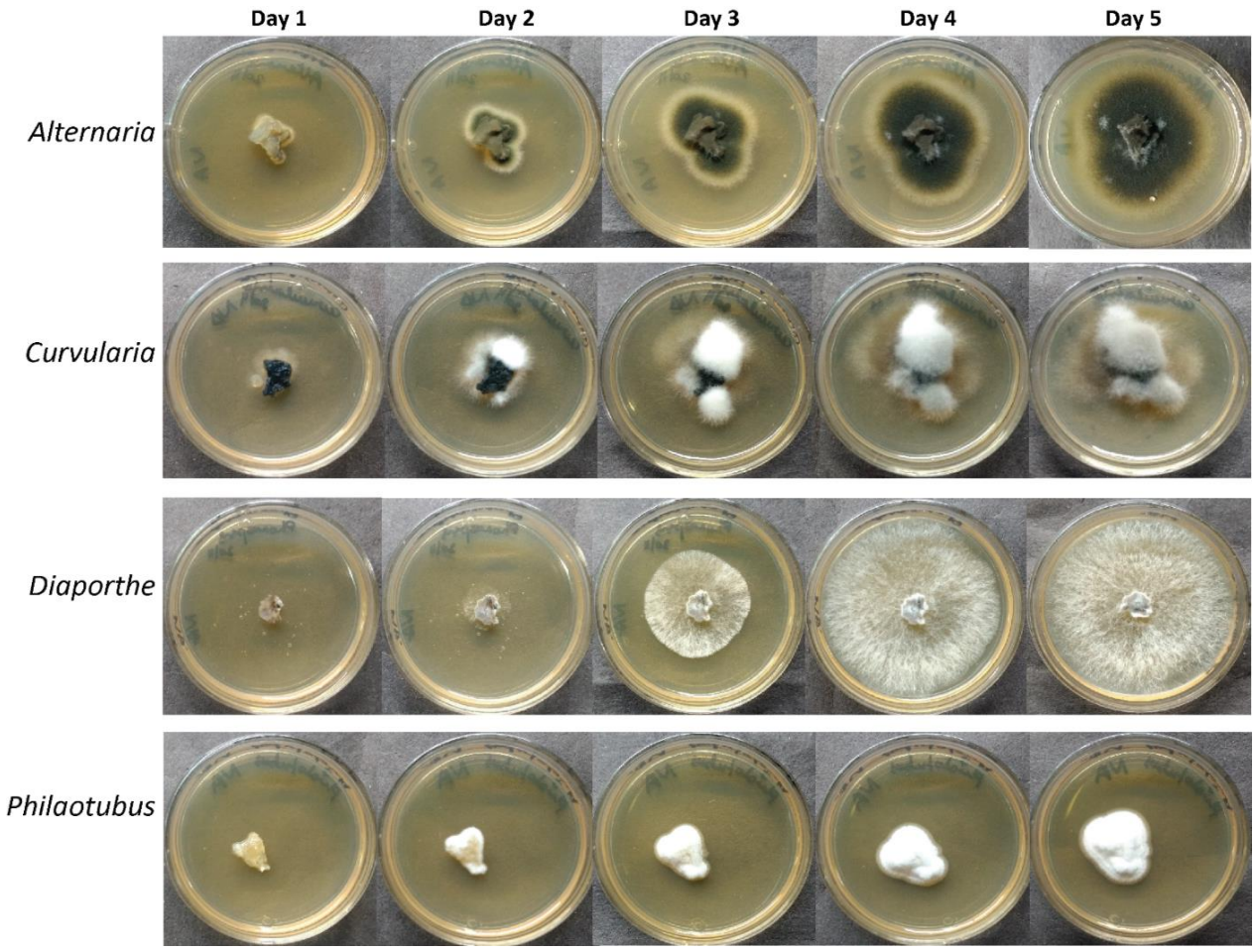


Fig. S14: Representative pictures of few fungi grown on previously used media from the interaction assay (test plates) that were re-plated. A simple explanation for the inhibitory capabilities seen for both *Pseudoxylaria* and *Termitomyces* could be the depletion of nutrients in the PDA plates. However, control experiments using previously used media to grow these fungi indicate the presence of sufficient nutrients in these plates for adequate fungal growth

Fig. S15: *Bacillus*- MN908297 Vs Fungal contaminants

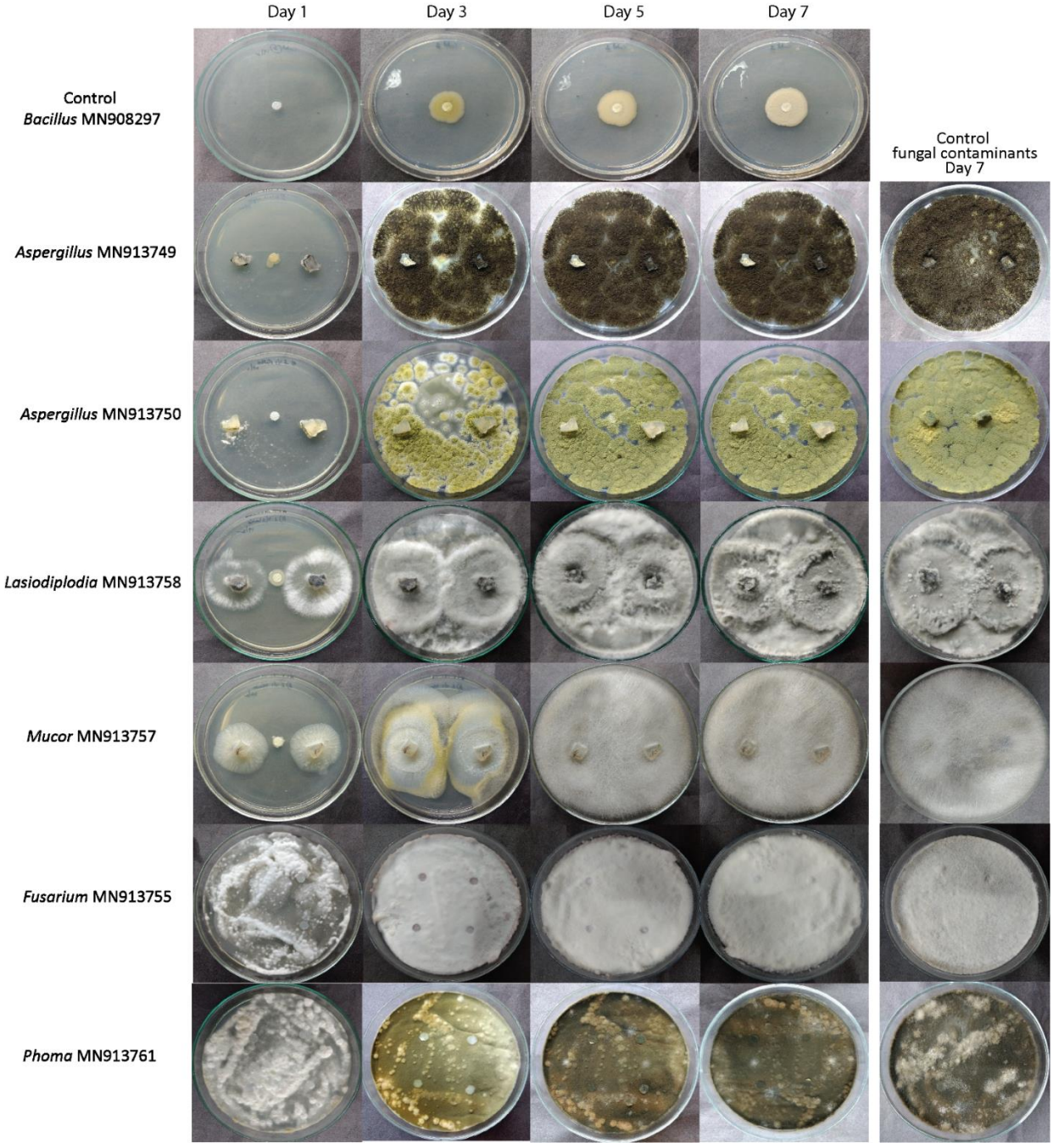


Fig. S15: Assay for the prevention of the growth of fungal contaminants by *Bacillus*- MN908297.

Fig. S16: *Bacillus*- MN908298 Vs Fungal contaminants

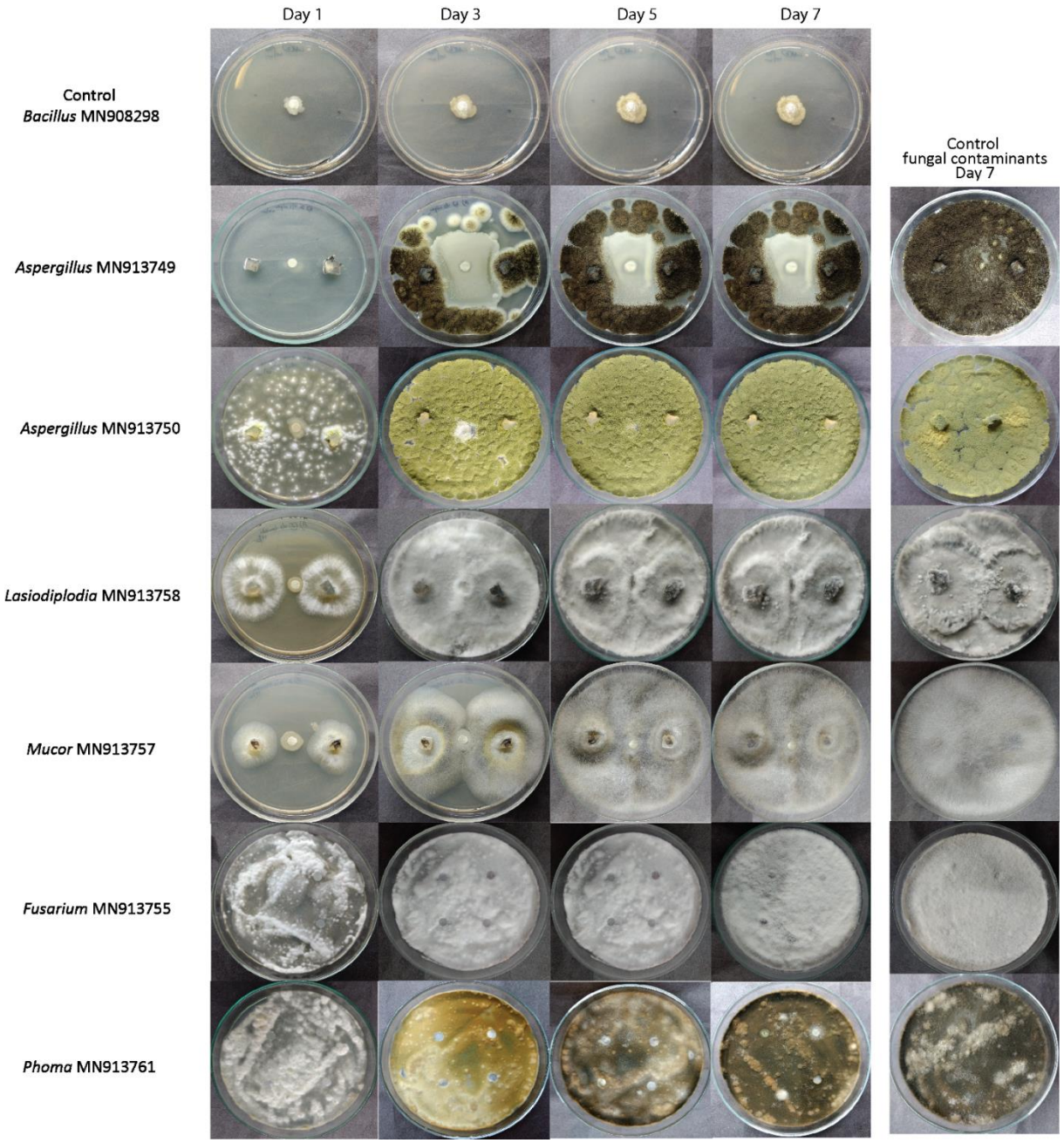


Fig. S16: Assay for the prevention of the growth of fungal contaminants by *Bacillus*- MN908298.

Fig. S17: *Bacillus*- MN908304 Vs Fungal contaminants

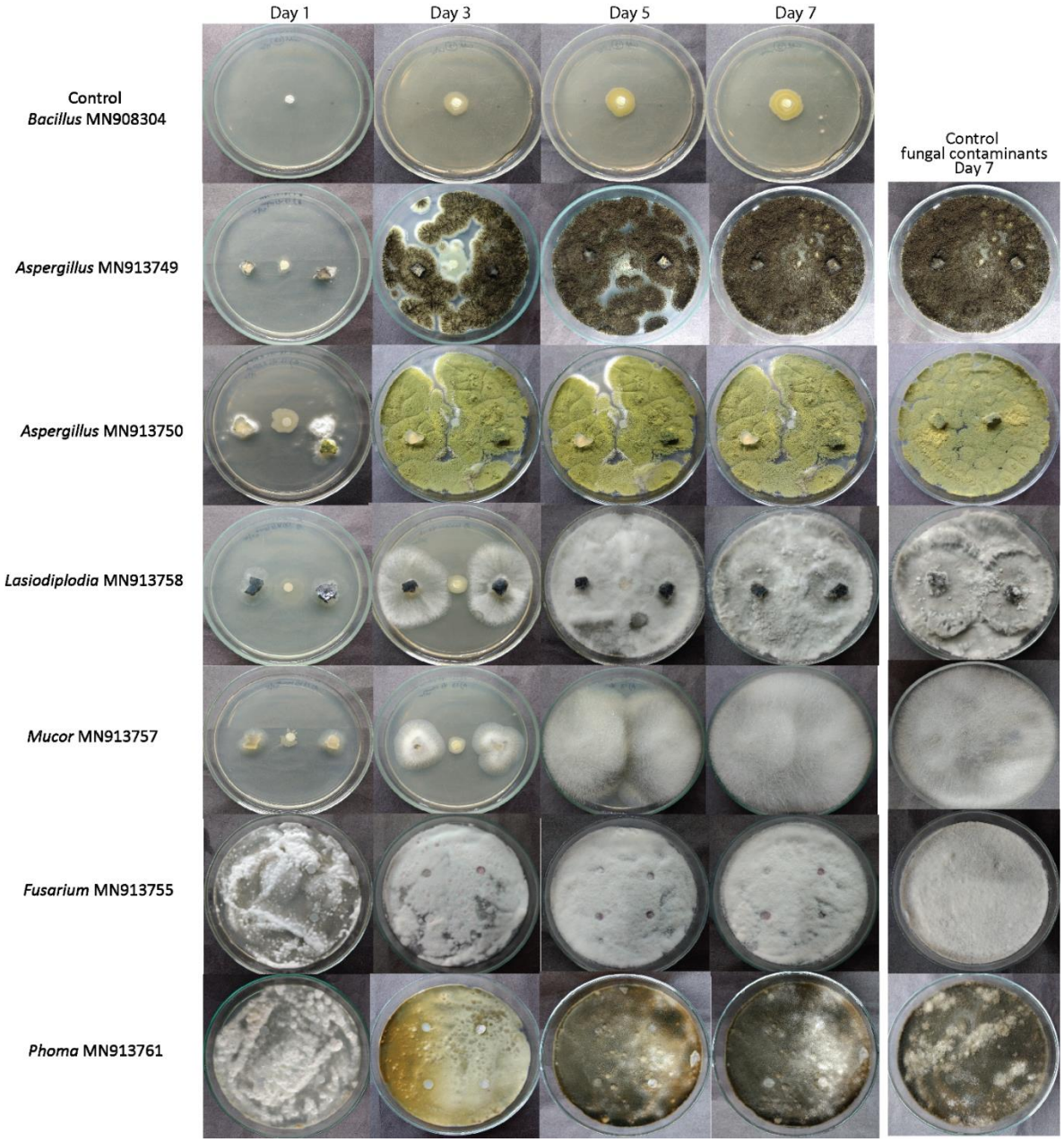


Fig. S17: Assay for the prevention of the growth of fungal contaminants by *Bacillus*- MN908304.

Fig. S18: *Bacillus*- MN908305 Vs Fungal contaminants

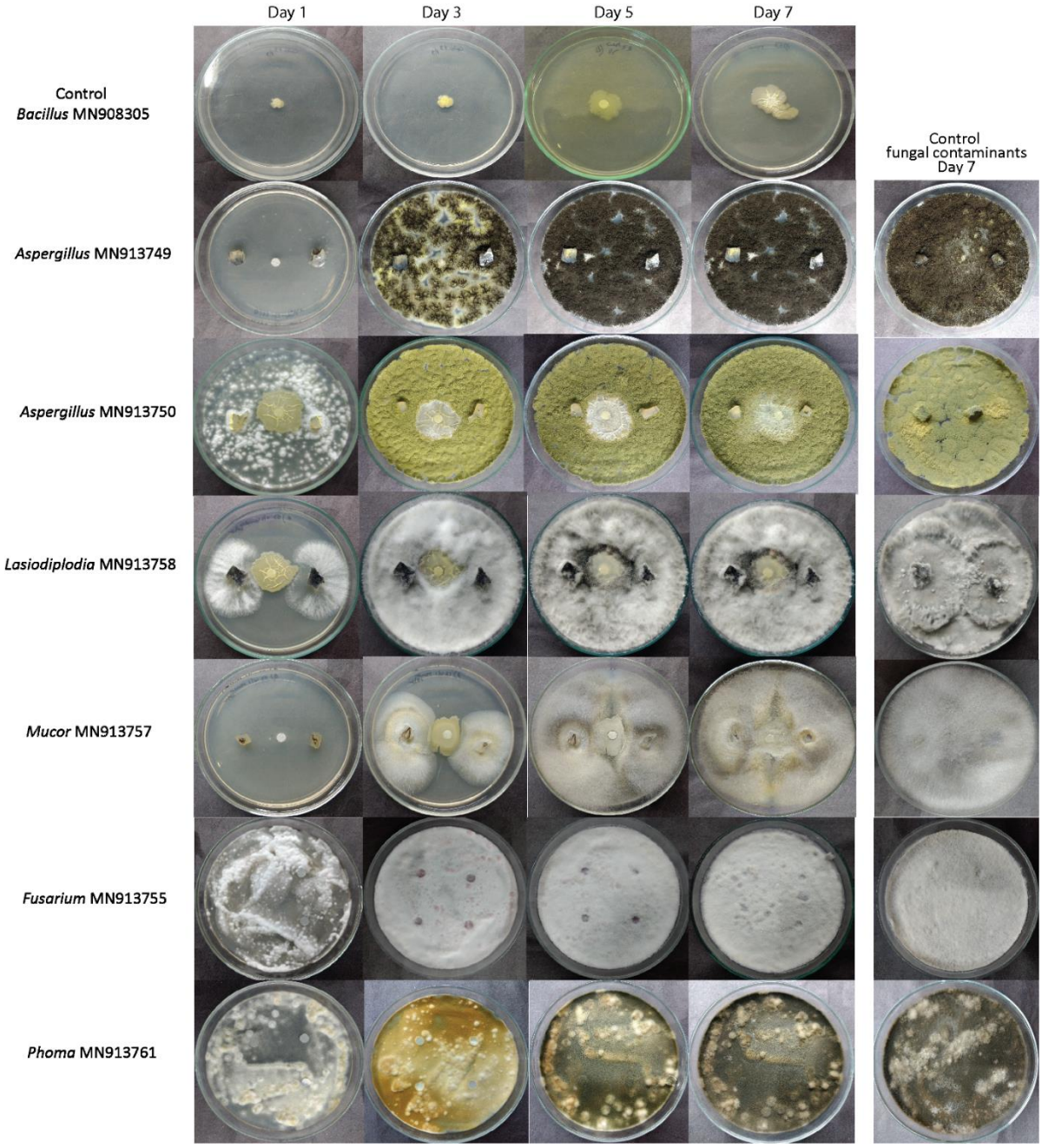


Fig. S18: Assay for the prevention of the growth of fungal contaminants by *Bacillus*- MN908305.

Fig. S19: *Burkholderia*- MN908310 Vs Fungal contaminants

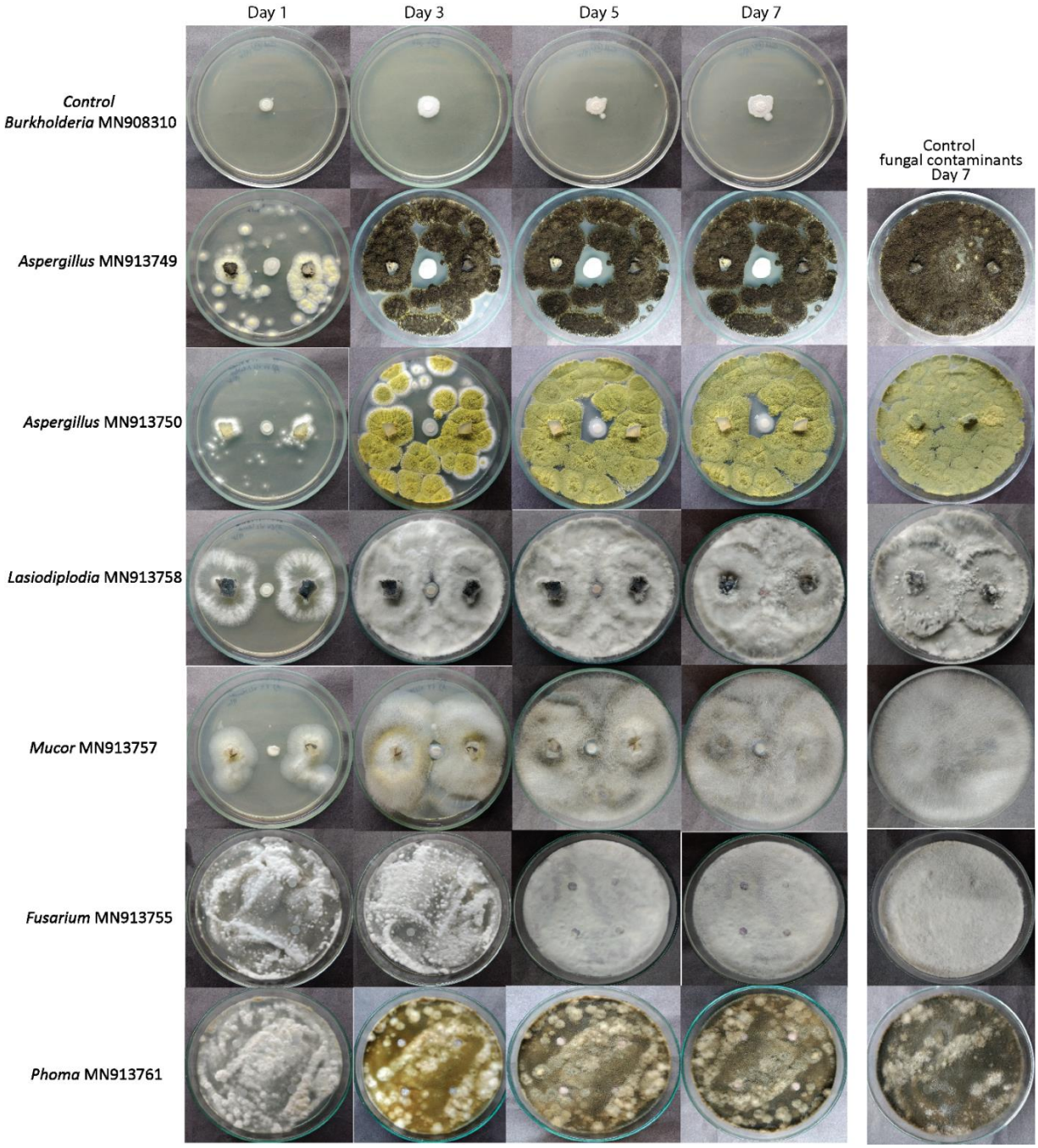


Fig. S19: Assay for the prevention of the growth of fungal contaminants by *Burkholderia*- MN908310.

Fig. S20: *Pseudomonas*- MN908321 Vs Fungal contaminants

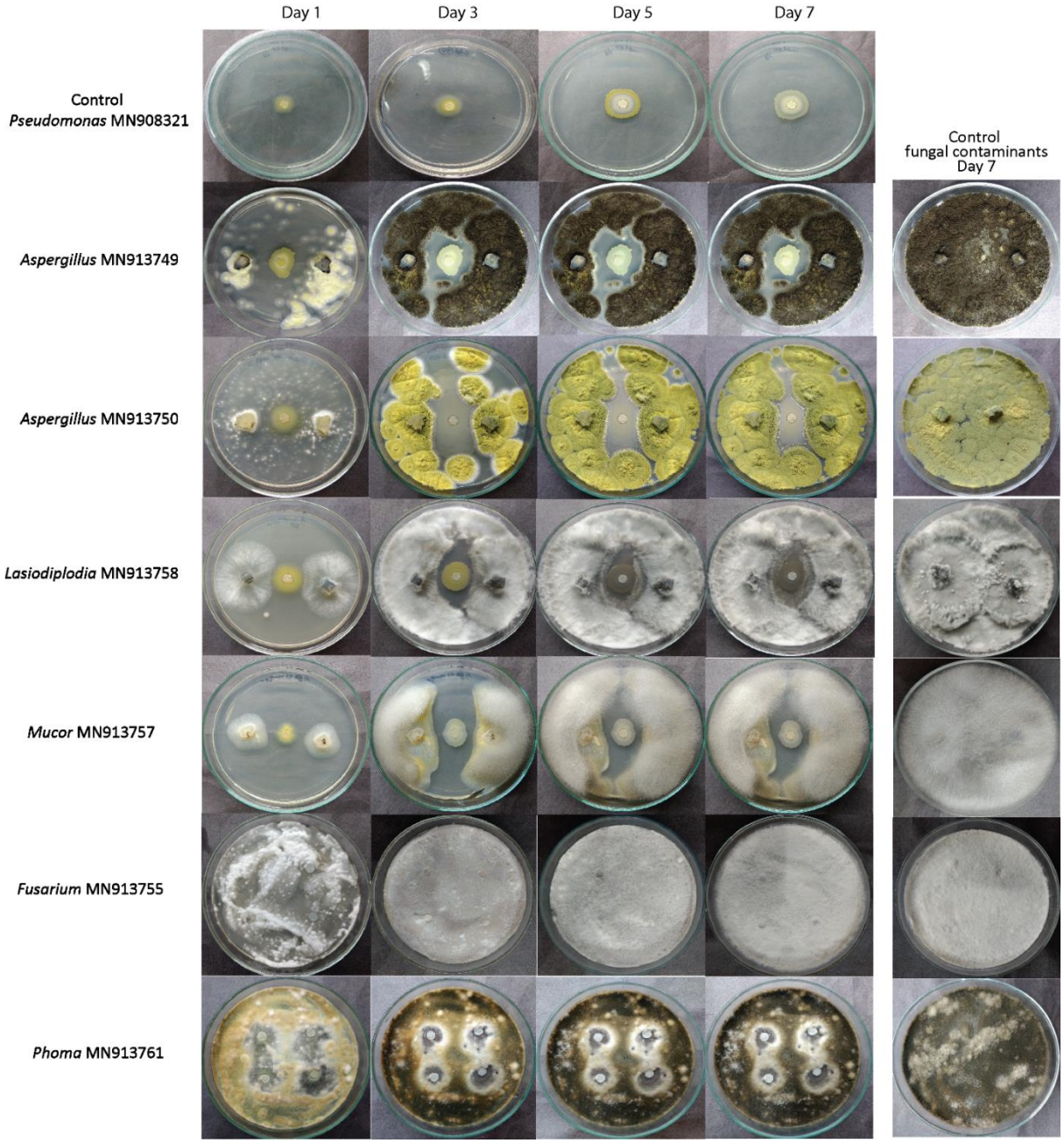


Fig. S20: Assay for the prevention of the growth of fungal contaminants by *Pseudomonas*- MN908321.

Fig. S21: *Pseudomonas*- MN908322 Vs Fungal contaminants

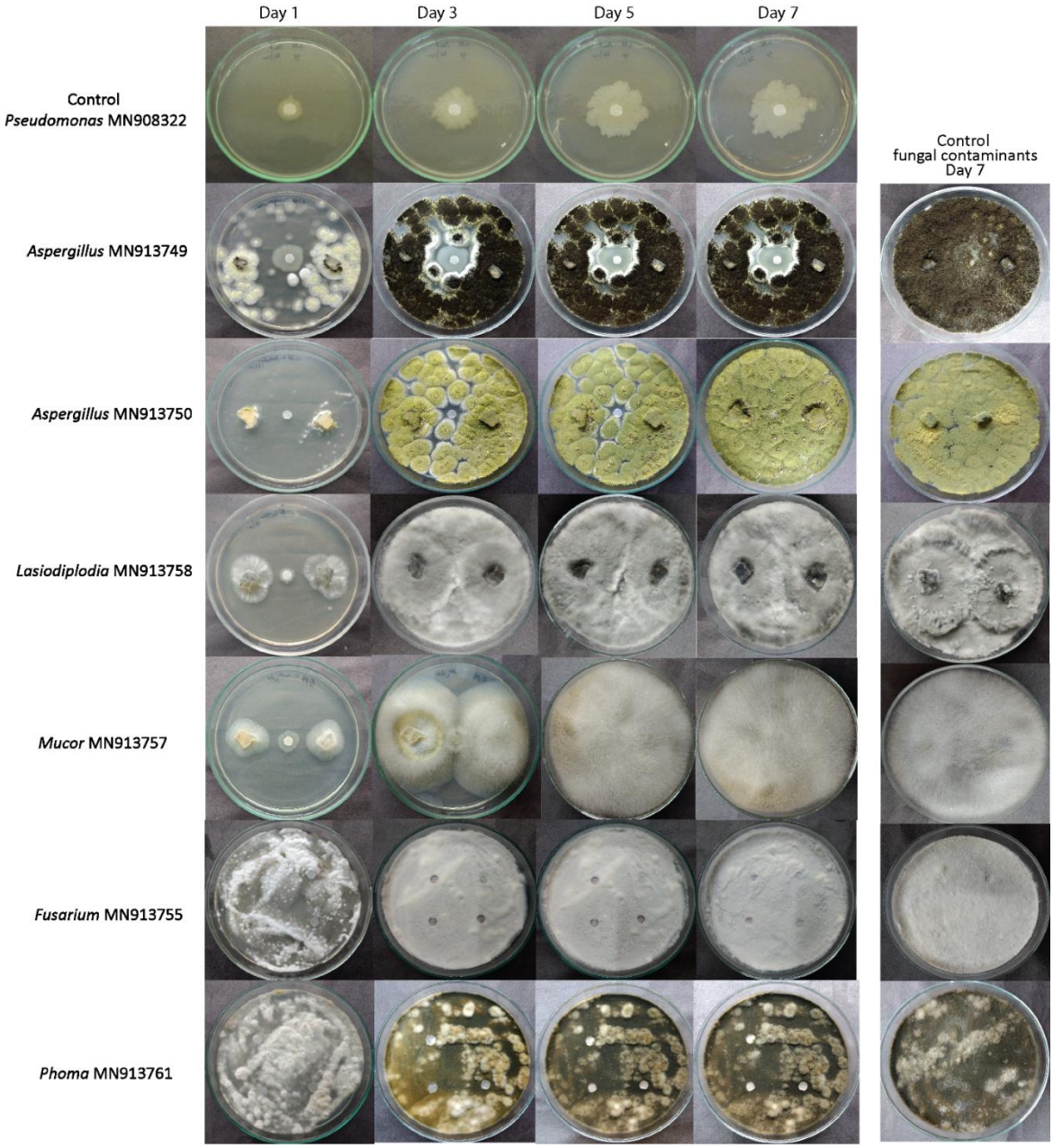


Fig. S21: Assay for the prevention of the growth of fungal contaminants by *Pseudomonas*- MN908322.

Fig. S22: *Streptomyces*-MN908327 Vs Fungal contaminants

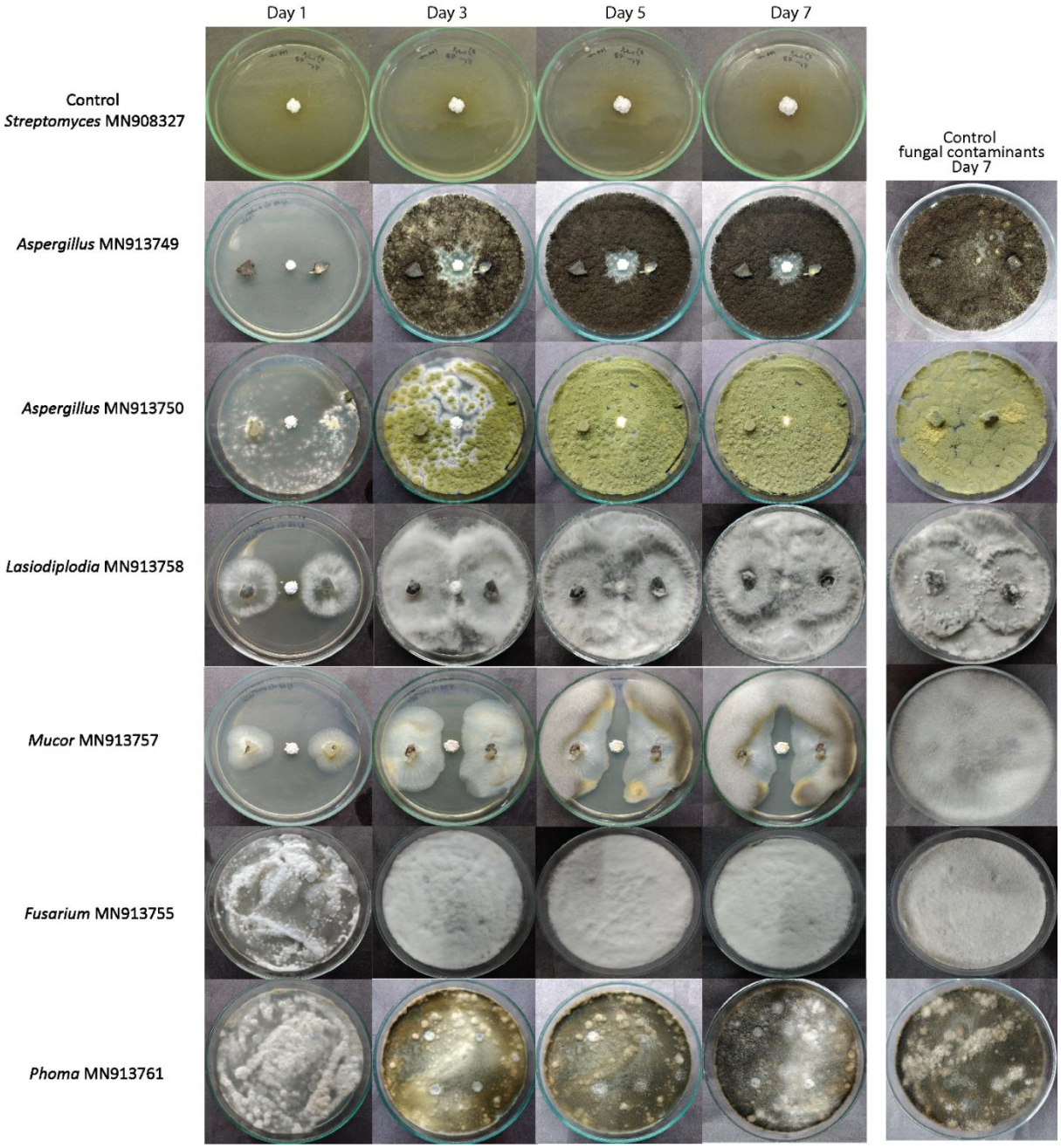


Fig. S22: Assay for the prevention of the growth of fungal contaminants by *Streptomyces*-MN908327.

Table S1: Details of the fungal strains isolated for the culture-dependent assay.

Isolated cultures (Genus-Accession no.)	Isolated from	Nearest neighbor in GenBank	% identity
Aspergillus sp. **(MN913749)**	Alate, Comb	KX928746	99.66
Aspergillus sp. **(MN913750)**	Alate	KU866665	100
Alternaria sp. **(MN913751)**	Alate	KT192402	100
Curvularia sp. **(MN913753)**	Alate	MN053866	100
Fusarium sp. **(MN913755)**	Comb	MW308555	100
Rhizomucor sp. **(MN913756)**	Alate, Comb	MT254752	99.83
Mucor sp. **(MN913757)**	Alate, Comb	MN853752	99.49
Lasiodiplodia sp. **(MN913758)**	Comb	MT302844	100
Paraconiothyrium sp. **(MN913759)**	Nymph	LT796893	100
Phialotubus sp. **(MN913760)**	Comb	JN831360	100
Phoma sp. **(MN913761)**	Alate	MT448809	99.42
Diaporthe sp. **(MN913762)**	Alate	KU663500	99.81
Syncephalastrum sp. **(MN913763)**	Alate	MK968099	98.95
Pseudoxylaria sp. **(MN913764)**	Comb	MK048540	100
Trichoderma sp. **(MN913767)**	Comb	MT530036	100
Termitomyces sp. **(MN913768)**	Comb	KR154979	100
Penicillium sp. **(MN913771)**	Comb	MT588795	99.82
Ustilago sp. **(MN913772)**	Alate	AY740168	99.74

Tabe S1: The fungal strains isolated in this study with the *Odontotermes obesus* colony part they were isolated from are listed. Molecular identification of each fungal strain is based on BLASTn results of ITS gene sequences. The list has the accession numbers of the top BLASTn hits for each of their ITS sequences and the percentage identity to these BLAST hits.

Table S2: The detailed information of termite castes used to study the mycobiota.

Samples ID	Biological replicates	Mound 1	Mound 2	Total replicates
Major worker	MaW1	+	+	6
	MaW2	+	+	
	MaW3	+	+	
Minor worker	MiW1	+	+	6
	MiW2	+	+	
	MiW3	+	+	
Nymph	Ny1	+	+	6
	Ny2	+	+	
	Ny3	+	+	
Male Alate	MA1	++		6
	MA2	++		
	MA3	++		
Female Alate	FM1	++		6
	FM2	++		
	FM3	++		

++ : Two technical replicates

Table S3: The detailed information of the degrading comb samples used to study the micro- and mycobiota.

Microbiota survey							
Comb Samples	Mound 1			Mound 2			Total Replicates/day
	August	September	October	August	September	October	
O hrs	+	+	+	+	+	+	6
24 hrs	+	+	+	+	+	+	6
48 hrs	+	+	+	+	+	+	6
72 hrs	+	+	+	+	+	+	6
96 hrs	+	+	+	+	+	+	6
120 hrs	+	+	+	+	+	+	6
Mycobiota survey							
Comb Samples	Mound 1			Mound 2			Total Replicates/day
	August	September	October	August	September	October	
O hrs	+	+	+	+	+	+	6
24 hrs	+	+	+	+	+	+	6
48 hrs	+	+	+	+	+	+	6
72 hrs	+	+	+	+	+	+	6
96 hrs	+	+	+	+	+	+	6
120 hrs	+	+	+	+	+	+	6

Table S4: List of primers used in this study

Primer name	Function	Samples amplified	Target	Sequence (5′→3′)	Annealing Temperature (°C)
Termito_F1	Forward	Pure culture	*Termitomyces*	CATCGTTTTCAACCACCTGTGC	60
Termito_R1	Reverse			GCCTATCCAAGCTCACAAAAGC	
Xyla_F1	Forward	Pure culture	*Pseudoxylaria*	GGCCCTGAAAACTCTGTTTCG	56
Xyla_R1	Reverse			GCCCAGCTGCAGACGAGAATA	
Pseudo_F1	Forward	Pure culture	*Pseudomonas*	GAGTAATGCCTAGGAATCTGC	56
Pseudo_R1	Reverse			CAGTATCAGTCCAGGTGGTCG	
qTermito_F	Forward	Fungus comb	*Termitomyces*	TCAACCACCTGTGCACCTTTTG	51
qTermito_R	Reverse			GCATAGACCGGAAATGCAG	
qXyla_F	Forward	Fungus comb	*Pseudoxylaria*	TCGTTGCTTAGCGTTGGGAGC	61
qXyla_R	Reverse			CTAGAGCGTGAAACCGACTC	
qPseudo_F1	Forward	Fungus comb	*Pseudomonas*	TCAACCTGGGAACTGCATCCA	60
Pse686R[11]	Reverse			ACACAGGAAATTCCACCACCC	

Table S5: The mycobiota degrading fungus comb of *O. obesus*. The table also shows different diversity indices for all the samples.

Sample source	Total read (initial)	Total reads (post-cleanup)	Reads identified	Fungal genera identified	Chao1	Shannon	Simpson	InvSimspon
Decaying comb (0 hrs)	$0.58x10^5$	$0.54x10^5$	$0.54x10^5$	111	199.3	0.53	0.20	1.25
Decaying comb (24 hrs)	$0.62x10^5$	$0.60x10^5$	$0.58x10^5$	83	116	0.47	0.17	1.20
Decaying comb (48 hrs)	$0.16x10^5$	$0.18x10^5$	$0.14x10^5$	103	157.4	1.67	0.75	4.04
Decaying comb (72 hrs)	$0.16x10^5$	$0.17x10^5$	$0.14x10^5$	110	152.5	1.97	0.80	5.24
Decaying comb (96 hrs)	$0.17x10^5$	$0.18x10^5$	$0.15x10^5$	99	184	1.54	0.70	3.43
Decaying comb (120 hrs)	$0.04x10^5$	$0.04x10^5$	$0.04x10^5$	71	126.1	1.47	0.63	2.73
				236 unique fungal genera				

Table S6: Number of copies of *Termitomyces, Pseudoxylaria and Pseudomonas* in different degrading stages of fungus comb using qPCR estimation. The highlighted cells are number of copies within 95% Confidence intervals.

		No. of copies/ ul		
	Samples	*Termitomyces*	*Pseudoxylaria*	*Pseudomonas*
0 hrs	Comb_1	1360107542	89981	175388
	Comb_2	1527079637	261070	120571
	Comb_3	3010663121	1662542	109453
	Comb_4	29961793	480150	16482
	Comb_5	1379035572	550258	12115
	Comb_6	66598339	965224	454345
	Comb_7	3076097533	10680	5262
	Comb_8	573685238	106523	868021
	Comb_9	703381182	200563	334247
	Comb_10	408063859	198522	1016288
	Comb_11	3019443861	N/A	6140510
24 hrs	Comb_1	1439414705	9648600	6059579
	Comb_2	533834327	2797119	64542
	Comb_3	2337609707	3912787	210228
	Comb_4	682213164	5980566	113264
	Comb_5	1456291250	8215710	165481
	Comb_6	44139049	7859628	1527
	Comb_7	984861560	2525442	3716
	Comb_8	1210550436	12659094	13237
	Comb_9	991863875	13882737	232467
	Comb_10	1516168056	8318347	218101
	Comb_11	1224549561	38050364	69040
	Comb_12	1605096212	27805362	1331129
48 hrs	Comb_1	2775237	933660096	69281
	Comb_2	695691	752072080	76725
	Comb_3	9064272	462970	58965
	Comb_4	8440967	180809717	73434
	Comb_5	195786877	23597478	12983
	Comb_6	13193507	3003351792	43232
	Comb_7	3787981	1824323516	697610
	Comb_8	137221322	892522016	268384
	Comb_9	11569396	185587389	986642
	Comb_10	30363382	2047154667	264786
	Comb_11	5539178	99217932	110009
	Comb_12	3968439	N/A	742690

72 hrs	Comb_1	735628	17242698	68775
	Comb_2	1252119357	1083222891	445587
	Comb_3	249477	7752681	149178
	Comb_4	1504145	201581	56405
	Comb_5	1874901	445655	6115877
	Comb_6	75413343	2824057	11582345
	Comb_7	5363423	4306131	27078
	Comb_8	462079025	8967365	14130
	Comb_9	37516583	358315851	3659116
	Comb_10	4398251	2942430	467746
	Comb_11	551510	130507319	135188
	Comb_12	276314	135926460	11997329
96 hrs	Comb_1	28614	3940039	50105
	Comb_2	85917	54880292	3873646
	Comb_3	2703112	116695	53624
	Comb_4	1430558	121515648	183775
	Comb_5	583616597	945957	57043
	Comb_6	1996	965994	17790
	Comb_7	197580	152511231	24126
	Comb_8	158590	13736078	8492
	Comb_9	346307	31014431	88413
	Comb_10	1200402	5246721	22053078
	Comb_11	137577	287839668	675092
	Comb_12	182311		173260
120 hrs	Comb_1	24967	3555896	113598
	Comb_2	75815	9409891	355757
	Comb_3	650499	559521	117656
	Comb_4	90228	15718962	32026
	Comb_5	632982	658760	85201
	Comb_6	1440370	5423933	1139999
	Comb_7	294558	10737477	139542
	Comb_8	2440949	12765611	61357038
	Comb_9	194647	1416038	164270
	Comb_10	582163	1182903	175860
	Comb_11	942411	26059452	153213
	Comb_12	488913	N/A	151121

Table S6 continued

Table S7: The microbiota of the degrading fungus comb of *O. obesus*. The table also shows different diversity indices for all the samples.

Sample source	Total reads (initial)	Total reads (post-cleanup)	Reads identified	Bacterial genera identified	Chao1	Shannon	Simpson	InvSimspon
Decaying comb (0 hrs)	$0.14x10^5$	$0.12x10^5$	$0.12x10^5$	587	587	4.4	0.9	32.0
Decaying comb (24 hrs)	$0.94x10^5$	$0.88x10^5$	$0.16x10^5$	390	390	3.7	0.9	19.9
Decaying comb (48 hrs)	$0.38x10^5$	$0.50x10^5$	$0.36x10^5$	2014	2014	6.0	0.9	137.4
Decaying comb (72 hrs)	$1.54x10^5$	$1.45x10^5$	$0.16x10^5$	374	374	3.7	0.9	22.7
Decaying comb (96 hrs)	$0.63x10^5$	$0.59x10^5$	$0.20x10^5$	298	298	3.3	0.9	13.9
Decaying comb (120 hrs)	$1.33x10^5$	$1.25x10^5$	$0.16x10^5$	278	278	3.4	0.9	15.4
				2, 182 unique bacterial genera				

Table S8: Inhibition efficiency of *Termitomyces*, *Pseudoxylaria* and bacterial mutualists on fungal contaminants.

Fungi ϯ | Bacteria ϕ

ϯ >50%: Efficient inhibition; <50%: Inefficient inhibition

Inhibition activity of fungi/ bacteria	***Termitomyces* MN913768**	***Pseudoxylaria* MN913764**	***Bacillus* MN908297**	***Bacillus* MN908298**	***Bacillus* MN908304**	***Bacillus* MN908305**	***Burkholderia* MN908310**	***Pseudomonas* MN908321**	***Pseudomonas* MN908322**	***Streptomyces* MN908327**	**Overall inhibition**
Aspergillus sp. **(MN913749)**	<50%	<50%	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Aspergillus sp. **(MN913750)**	<50%	<50%	No	No	Yes	Yes	Yes	Yes	No	No	Yes
Alternaria sp. **(MN913751)**	>50%	>50%									Yes
Curvularia sp. **(MN913753)**	>50%	<50%									Yes
Fusarium sp. **(MN913755)**	<50%	<50%	No	No	No	No	No	No	No	No	No
Rhizomucor sp. **(MN913756)**	Not clear	>50%									Yes
Mucor sp. **(MN913757)**	No	<50%	No	No	No	No	Yes	Yes	No	Yes	Yes
Lasiodiplodia sp. **(MN913758)**	<50%	<50%	No	No	Yes	No	No	Yes	No	No	Yes
Paraconiothyrium sp. **(MN913759)**	<50%	<50%									No
Phialotubus sp. **(MN913760)**	<50%	<50 %									Yes
Phoma sp. **(MN913761)**	<50%	<50%	No	No	No	No	No	Yes	No	No	Yes
Diaporthe sp. **(MN913762)**	Not Clear	>50%									Yes
Syncephalastrum sp. **(MN913763)**	>50%	>50%									Yes
Trichoderma sp. **(MN913767)**	No	>50%									Yes
Penicillium sp. **(MN913771)**	<50%	>50%									Yes
Ustilago sp. **(MN913772)**	>50%	>50%									Yes

ϕ Yes: inhibition; No: no inhibition

Overall inhibition: Inhibition by any of the fungi or bacteria

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