

Analysis of the nasal vestibule mycobiome in patients with allergic rhinitis

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Summary

Advances in culture-independent sequencing methods have been utilised in recent studies to understand the phylogenetic composition of the human microbiome of healthy and diseased skin. Allergic rhinitis (AR) is an inflammatory condition of the nasal cavity caused by environmental allergens. Although nasal microbial communities have been considered important contributors in human health, no studies to date have comprehensively compared fungal communities (mycobiome) of the nasal vestibule using the culture-independent pyrosequencing method. This study aimed to investigate how fungal communities of the nasal vestibule skin surface are influenced by AR. The phylogenetic composition of the nasal vestibule mycobiome of patients with AR was analysed by culture-independent pyrosequencing methods and compared with healthy individuals. A total of 69 fungal genera were identified from both AR samples and healthy controls, and the genus *Malassezia* predominated in the nasal vestibule. Species-level analysis classified eight different *Malassezia* species including *M. pachydermatis* and *M. cuniculi*, which were normally isolated from animals, and revealed *M. restricta* to be the most abundant species in the nasal vestibule. Although high interpersonal variation was observed, some of the AR samples displayed significantly higher diversities than healthy controls at both the genus and species level.

Key words: Allergic rhinitis, nasal vestibule, mycobiome, fungal communities, *Malassezia*.

Introduction

Allergic rhinitis (AR) is an inflammatory condition of the nasal mucosa and is mediated by environmental allergens such as pollen, animal dander and mould.^{1,2} Characteristics of AR include infiltration of the nasal mucosa with inflammatory cells including mast cells, eosinophils, basophils and T lymphocytes. AR is considered a systemic inflammatory disorder and is often

associated with asthma, rhinosinusitis, allergic conjunctivitis and atopic dermatitis; both AR and asthma are known to share common immunopathological mechanisms characterised by type 2 helper T-cell-dependent inflammation, and patients with AR show a significantly higher rate of respiratory infections.^{3,4}

Recent advances in culture-independent sequencing technologies have enabled the determination of microbiomes of various sites of the human body, including skin.^{5–7} Microbiome characterisation provides insight into the differences between healthy and diseased skin, such as psoriasis and atopic dermatitis. A recent study revealed that bacterial communities of the skin in healthy individuals are composed of four predominant phyla: Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%) and Bacteroidetes (6.3%). At the species level, *Propionibacteria* and *Staphylococci*

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predominated in sebaceous sites and *Corynebacteria* predominated in moist sites, while mixed species were found in dry sites.⁸ Bacterial communities in healthy human nasal cavity were also analysed by the culture-independent sequencing method and were shown to be dominated exclusively by two phyla, Actinobacteria and Firmicutes (85.4–95.0%).^{9,10}

The human skin microbiome consists not only of bacteria; fungi also play a significant role in the stability of microbial communities in human skin.^{11,12} The culture-independent pyrosequencing method has been applied to understand fungal community (mycobiome) structures of the human skin. Recently, Findley *et al.* analysed the phylogenetic composition of the human skin mycobiome using the culture-independent pyrosequencing method and reported that the genus *Malassezia* exclusively predominates in almost all sites tested. In particular, *M. restricta* and *M. globosa* were the two most abundant species, with the former specifically predominating in the external auditory canal, retroauricular crease and glabella, while the latter predominated on the back, occiput and inguinal crease.¹³ Oh *et al.* also observed elevated fungal diversity as well as increased detection of opportunistic fungi such as *Candida* and *Aspergillus* in immunodeficient patients, which suggests that the fungal community is altered by host immunity and may play an underlying role in skin disease.¹⁴

Although nasal mycobiome have been analysed and considered important contributors in human health, previous studies have relied on classical culture-dependent methods and incompletely defined community structures.^{15,16} No studies to date have comprehensively compared mycobiome of the nasal vestibule using the culture-independent pyrosequencing method. In this study, we investigated the influence of AR on the nasal mycobiome, mainly focusing on fungal communities on the nasal vestibule skin surface, which is lined with keratinising squamous epithelium and is the most anterior part of the nasal cavity, in patients with AR compared to healthy individuals. We reasoned that the existence of certain fungal species in the nasal vestibule may influence AR because the nasal cavity can be more frequently exposed to it.

Materials and methods

Sample collection

All samples in this study were obtained according to a protocol approved by the Institutional Review Board (IRB) of Ethics Committee of Konkuk University

Medical Center (KUH1120037). All human participants, AR patients and healthy controls, were from the Seoul metropolitan region in Korea and provided the IRB-approved written informed consent to be enrolled in this study. To collect samples, swabs were washed with phosphate-buffered saline containing 0.1% Triton ×100. The nasal vestibule of each subject was swabbed 10 times, and DNA was isolated as described previously.¹⁷

PCR amplification and pyrosequencing

PCR amplification was performed using barcoded primers targeting the D1/D2 region of the large ribosomal subunit (*LSU*) gene (Table S1). Amplifications were performed using extracted DNA under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final elongation at 72 °C for 5 min. PCR products were confirmed by 2% agarose gel electrophoresis and visualised under a Gel Doc system (Bio-Rad, Hercules, CA, USA). The amplified products were purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Equal concentrations of purified products were pooled together, and short fragments (non-target products) were removed using an Ampure beads kit (Agencourt Bioscience, MA, USA). The quality and product size were assessed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Mixed amplicons were prepared using emulsion PCR and then deposited on Picotiter plates. Sequencing was carried out at Chunlab, Inc. (Seoul, Korea), using the GS Junior Sequencing system (Roche, Branford, CT, USA) according to the manufacturer's instructions. We de-noised and quality filtered the raw 454 SFF files using the mothur software package v. 1.32.1.¹⁸

Pyrosequencing data analysis and *Malassezia* species classifications

Reads exceeding 250 bp were assigned to a fungal genera using the naive Bayesian rRNA classifier RDP classifier v. 2.5.¹⁹ Reference *LSU* sequences used for matching were hosted by the Ribosomal Database Project (RDP 11 Database Update 1; accessed November 2013).²⁰ We retained taxonomic assignments if the confidence estimate by bootstrap exceeded 90%. We retrieved all sequences attributed to the *Malassezia* genus by the RDP classifier to assign species-level identities, creating a custom database of *Malassezia LSU* sequences. We queried Genbank (accessed November

2013) to identify sequences matching the following search terms: *Malassezia*[Organism] AND (25S OR 26S OR 28S OR “large ribosomal subunit”) NOT (mitochondrial OR mitochondrion OR sp.[TITL] OR cf.[TITL] OR uncultured[TITL] OR environmental[TITL]). We manually inspected the query results for inconsistencies and excluded sequences that did not span at least 95% of the amplicon sequence. Database sequences were aligned with MAFFT using the maxiterate 1000 option.²¹ We used the mothur command *align.seqs* to align all 454 amplicon sequences assigned to the *Malassezia* genus against the custom *Malassezia* LSU database. Species identities were assigned with the mothur command *classify.seqs* using the kmer-based Wang method. We computed 1000 bootstrap iterations to assign species probabilities and retained assignments with a bootstrap support above 80%.

Phylogenetic diversity analyses

We used custom scripts in R and *ggplot2* to organise and visualise the species distribution data.^{22,23} Principal component analysis (PCA) was performed using the R *stats* package.²² The diversity index was calculated using the R packages *vegan*.²⁴

Results

AR diagnosis was defined by the 2008 Joint Task Force of Practice Parameters.²⁵ None of the subjects had taken any medication for at least four weeks prior to the fungal community analysis. To analyse fungal communities, swabs were prepared from the nasal vestibule of nine AR patients and six healthy individuals (healthy controls). DNA was extracted from each swab, and the LSU D1/D2 region was amplified by PCR as described above. Among the specimens, five and two samples from AR patients and healthy controls were excluded, respectively, due to negative PCR results, which may have been caused by insufficient template DNA. The remainder of the samples (four AR samples and four healthy controls), which showed positive PCR results, were included in the analysis (Table 1).

We first determined the relative abundance of fungal phylum in the swab samples. Basidiomycota was highly abundant, at 97.56–99.99% in AR samples and 99.06–99.76% in healthy controls, and Ascomycota represented a minor proportion, at 0.01–2.44% and 0.24–0.94% in AR samples and healthy controls respectively. Only one AR sample (A4) contained Chytridiomycota (Fig. 1a). No significant differences

Table 1 Clinical status of samples and number of generated sequence reads.

Sample ID	Age	Gender	Allergic rhinitis	Number of sequence reads
A1	23	Female	Yes	1,210
A2	42	Male	Yes	13,347
A3	22	Female	Yes	4,407
A4	27	Female	Yes	14,159
H1	28	Male	No	6,884
H2	39	Female	No	2,853
H3	27	Female	No	10,973
H4	31	Female	No	9,842

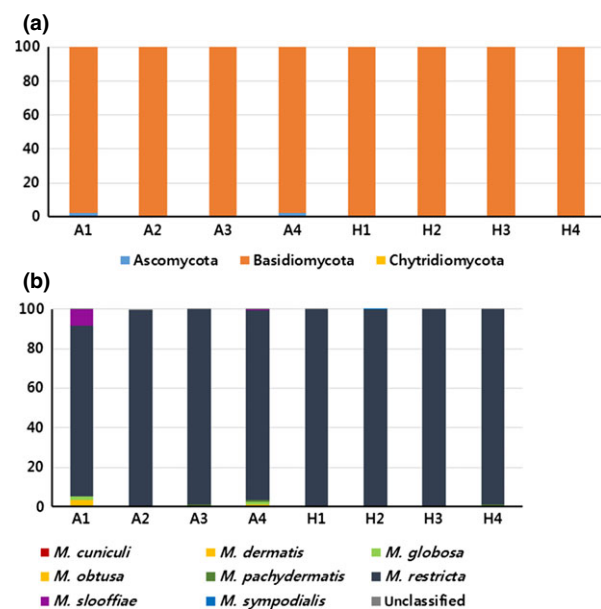


Figure 1 Relative abundance of fungal phyla and *Malassezia* species. Large ribosomal subunit (LSU) sequences of AR samples (A1–A4) and healthy controls (H1–H4) were taxonomically classified to the phylum (a) and the species level (b). The relative abundance of fungal phyla or species in each sample was expressed as a percentage (Y-axis).

between AR samples and healthy controls were seen at the phylum level. When we explored the diversity at class, order and family levels, Exobasidiomycetes (91.51–99.93% in AR samples and 95.01–98.37% in healthy controls), Malasseziales (91.05–99.93% in AR samples and 95.17–98.47% in healthy controls) and Malasseziaceae (91.30–99.95% in AR samples and 95.82–98.73% in healthy controls) were found to be the most abundant (Table S2–S4). However, as at the phylum level, no significant differences were seen between AR samples and healthy controls.

Previous studies have suggested that alteration of host immunity caused an imbalance in fungal communities typically detectable at the genus level, such as increased occurrence of *Aspergillus* and *Candida* genera in patients with primary immunodeficiency and more diverse fungal genera on the skin of patients who underwent antifungal therapy.^{13,14} In this study, we identified a total of 69 genera including common opportunistic pathogenic fungi *Aspergillus* and *Cryptococcus*. Airborne *Alternaria* spores have been associated with AR symptoms,²⁶ and the genus was identified from one of the AR samples, A3 (Table S5). The genus *Malassezia* highly predominated, at 92.03–94.17% and 96.20–98.79% in AR samples and healthy controls, respectively, followed by *Schizophyllum* and *Cryptococcus*. These results led us to analyse the species diversity of fungal communities focusing on the *Malassezia* genus.

Species-level analysis demonstrated that nasal vestibule fungal communities of AR patients and healthy controls are composed of at least eight *Malassezia* species: *M. restricta*, *M. globosa*, *M. sympodialis*, *M. slooffiae*, *M. dermatis*, *M. pachydermatis*, *M. cuniculi* and *M. obtusa* (Fig. 1b). Among the 60,973 sequence reads belonging to the *Malassezia* genus, 59,901 were identified as *M. restricta*. More specifically, *M. restricta* represented 86.48–99.33% and 98.42–99.75% of the total sequence reads of AR samples and healthy controls respectively. These data suggest that *M. restricta* is the most predominant species in nasal vestibules of both AR samples and healthy controls (Table S6). *M. globosa* was found in all samples and was the second most predominant species, representing 0.07–1.65% and 0.01–0.74% of the total sequence reads of AR samples and healthy controls respectively. *M. pachydermatis* is considered to be zoophilic, normally colonising the skin and ears of domestic animals such as dogs, and causes otitis and dermatitis. *M. pachydermatis* occasionally infects humans and has emerged as an important pathogenic yeast responsible for systemic infections in neonates in intensive care units.²⁷ Interestingly, we found that minor sequence reads (up to 1.02%) from all samples except one healthy control (H1) contained *M. pachydermatis*. However, no correlation between *M. pachydermatis* and AR was found in this study. *M. cuniculi* was recently described and isolated from rabbit skin.²⁸ Among the samples, only one AR sample (A4) contained *M. cuniculi*. The same sample also contained sequence reads of *M. obtusa*, which was not found in any other sample. Both *M. cuniculi* and *M. obtusa* were only identified in the A4 sample, suggesting that the nasal vestibule of this particular patient possesses the most diverse fungal community.

We next compared fungal community diversity of AR samples with that of healthy controls at both genus and species levels using the Shannon diversity index (SDI), a common weighted measure which has been used to evaluate both richness and evenness of microbial communities in an ecological niche.²⁹ As shown in Fig. 2a, A1, A3 and A4 showed notably higher SDI than healthy controls at the genus level, among AR samples. However, one AR sample (A2) showed significantly low SDI suggesting that AR patients exhibit high interpersonal variation. Although the three most diverse fungal communities were found in AR samples, genus-level diversity analysis showed no statistical differences compared to healthy controls (Wilcoxon rank sum test, $W = 12$, $P = 0.3429$). We also determined species-level diversity using SDI. Among AR samples, A1 and A4 showed notably higher species-level diversity compared to healthy controls (Fig. 2b). These results suggest a possible correlation between increased fungal diversity and AR in the host environment, as observed at the genus level. Interpersonal variation among fungal communities of AR samples at the species level was also significantly higher than in healthy controls. Although some AR samples showed higher species-level diversity than healthy controls, the overall comparison between AR samples and healthy controls was not significant (Wilcoxon rank sum test, $W = 11$, $P = 0.486$). PCA were performed to compare the abundance of each *Malassezia* species, and showed that AR samples were considerably more differentiated in *Malassezia* fungal communities compared to healthy controls (Fig. 3).

In this study, the diversity of fungal communities in the nasal cavities of AR patients and healthy individuals was successfully compared using culture-independent sequencing methodology. *Malassezia* predominated with *M. restricta* the most prevalent species. Our data suggest that either fungal community imbalance is associated with AR or that AR causes alteration of fungal community structures in nasal vestibules of AR patients.

Discussion

Malassezia are obligatorily lipid-dependent species and considered normal cutaneous fungal flora of the skin of humans and other warm-blooded animals. Attention has been focused on the distribution and biology of the species due to their association with various skin diseases such as pityriasis versicolor, seborrhoeic dermatitis, dandruff and atopic dermatitis. To date, 14 species of *Malassezia* have been recognised.^{30–32}

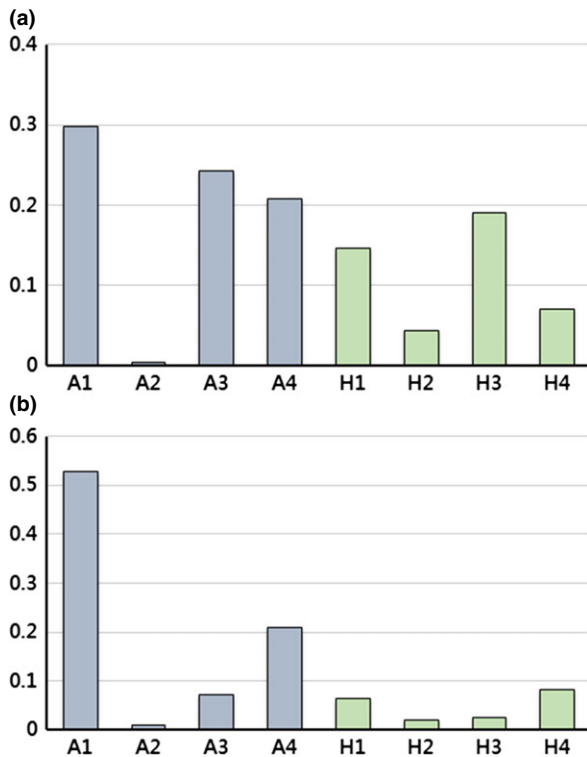


Figure 2 Comparison of diversity across samples. Shannon diversity indexes (SDI) of AR samples (A1–A4) and healthy controls (H1–H4) were determined. (a) Distribution of SDI values across samples at the genus level. (b) Distribution of SDI values across samples at the species level.

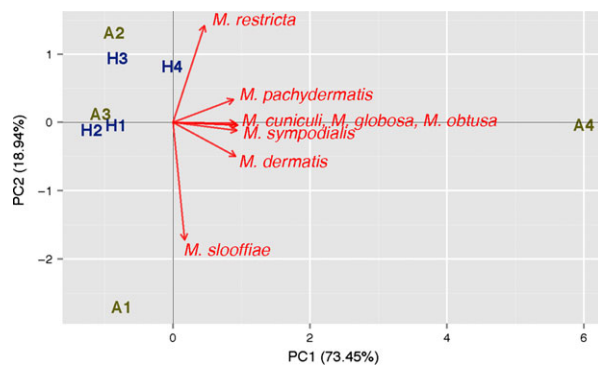


Figure 3 Principal component analysis (PCA) of fungal community similarity. Differences in the *Malassezia* fungal community structure among AR samples (A1–A4) and healthy controls (H1–H4) were analysed. The three major axes of PCA are shown with their respective percentage of explained variance. Arrows represent the associated *Malassezia* species.

Culture-independent sequencing methods confirmed a tight association of *Malassezia* species with various skin sites of the human body. Findley *et al.* explored

fungal communities of 11 body and arm sites (antecubital fossa, back, external auditory canal, glabella, hypothenar palm, inguinal crease, manubrium, nare, occiput, retroauricular crease and volar forearm) of healthy adults, and found that the genus *Malassezia* predominated in all sites investigated. Furthermore, at the species level, *M. restricta* predominated in the external retroauricular crease, auditory canal and glabella, while *M. globosa* predominated on the back, occiput and inguinal crease. It was also suggested that fungal infection is associated with fungal community imbalance and that antifungal treatment may cause alterations of skin fungal communities.¹³

In this study, we report that *Malassezia* predominated in the nasal vestibule skin surface of both AR patients and healthy individuals. We identified a total of eight different *Malassezia* species from the nasal vestibule skin surface and found *M. restricta* to be the most abundant species, similar to other human skin sites.^{13,14} Following *M. restricta*, *M. globosa* and *M. pachydermatis* were predominant, and *M. restricta*, *M. globosa*, *M. dermatitis* and *M. slooffiae* were found in all samples. *M. pachydermatis* was found in all samples except one healthy control (H1). *M. pachydermatis* is particularly interesting as this non-lipid-dependent species is not typically isolated from human skin sites due to its zoophilic nature. However, a previous study showed that *M. pachydermatis* can be easily transferred from dogs to immunocompromised patients by health-care workers who own dogs,³³ and several studies have reported that *M. pachydermatis* causes systemic infections in neonates in intensive care units.^{27,34} *M. sympodialis* was identified from only two AR samples (A1 and A4) and two healthy controls (H2 and H4). *M. cuniculi*, another zoophilic species isolated from rabbits, and *M. obtusa* were only found in one patient (A4).²⁸ Throughout the study, one of the AR samples (A4) showed the greatest diversity. However, we have no further information associated with the corresponding patient, such as residential history or presence of domestic animals. In addition to the above-mentioned A4 sample, A1 and A3 samples displayed increased genus-level diversity. Among the AR samples, A1 and A4 showed greater species-level diversity than healthy controls. These results suggest either that the nasal vestibule skin surface of AR patients may be more permissive than that of healthy individuals or that AR may alter the fungal diversity of the nasal vestibule. We also observed high interpersonal variation among AR samples, as observed in other studies.^{13,14,35} Although this study included a small number of subjects, which were not sufficient to fully understand

fungus communities in the nasal microenvironment, our data provide useful insights for future investigations.

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Conflict of interest

All the authors have declared that no competing interests exist.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Barcoded primers for PCR amplification.

Table S2. Relative proportion of fungal classes.

Table S3. Relative proportion of fungal orders.

Table S4. Relative proportion of fungal families.

Table S5. Relative proportion of fungal genera.

Table S6. Relative proportion of *Malassezia* species.

Table S1. Barcoded primers for PCR amplification

	Barcode	Forward primer	Reverse primer
A1	TGATGTGTAC	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
A2	CTGATGCT	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
A3	CTAGTGCT	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
A4	CTCATACAC	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
H1	TGCTATAGAC	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
H2	TACGCTCTCGT	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
H3	CATCATC	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC
H4	CAGTCTCGA	ACCCGCTGAAYTTAAGCATAT	CTTGGTCCGTGTTTCAAGAC

Table S2. Relative proportion of fungal classes

	A1*	A2	A3	A4	H1**	H2	H3	H4
Agaricomycetes	2.579035	0.059939	5.711035	0.050506482	1.061355	0.807018	4.505655	1.38507
Agaricostilbomycetes	0	0	0	0	0	0	0.018242	0
Ascomycota incertae sedis	0	0	0	0.00021251	0	0	0	0
Chytridiomycetes	0	0	0	7.08366E-05	0	0	0	0
Dothideomycetes	0.249584	0.007492	0.182025	0.007508677	0.029078	0.105263	0.264502	0.142581
Eurotiomycetes	0.083195	0	0.022753	0.00021251	0.523408	0.035088	0.009121	0
Exobasidiomycetes	91.51414	99.93257	93.97042	0.924559042	96.00174	98.35088	95.07479	98.37051
Lecanoromycetes	0	0	0	0	0	0	0.018242	0
Leotiomycetes	0	0	0.022753	0.002762627	0.014539	0	0.027362	0
Pezizomycetes	0	0	0	0	0	0	0	0.010184
Pneumocystidomycetes	0	0	0	0	0.014539	0	0	0
Pucciniomycetes	0	0	0	0.00021251	0	0	0	0
Saccharomycetes	0.249584	0	0	0.000354183	0.072696	0.070175	0	0.020369
Sordariomycetes	1.164725	0	0.068259	0.011546363	0.247165	0.526316	0.036483	0.061106
Tremellomycetes	4.159734	0	0.022753	0.002054261	1.991858	0.070175	0.045604	0.010184
Wallemiomycetes	0	0	0	0	0.043617	0.035088	0	0

* Allergic rhinitis sample; ** Healthy control

Table S3. Relative proportion of fungal orders

	A1*	A2	A3	A4	H1**	H2	H3	H4
Agaricales	0.531444	0.029969	2.393435	3.329056	0.320653	0.2106	1.972423	0.520408
Agaricostilbales	0	0	0	0	0	0	0.018263	0
Ascomycota incertae sedis	0	0	0	0.021386	0	0	0	0
Atheliales	0	0	0	0	0.014575	0.0351	0.027395	0
Auriculariales	0	0	0.022795	0	0	0	0.009132	0
Calosphaeriales	0	0	0	0.007129	0.02915	0	0	0
Cantharellales	0	0	0.068384	0.071286	0	0	0	0.010204
Capnodiales	0	0.007492	0.159562	0.399202	0.014575	0.1053	0.155237	0.071429
Chaetothyriales	0	0	0.022795	0	0.014575	0	0	0
Cystofilobasidiales	0	0	0	0	0.043725	0.0351	0	0
Diaporthales	0.354296	0	0.068384	1.083547	0.131176	0.4212	0.036526	0.05102
Dothideales	0	0	0	0.0499	0	0	0.100447	0.010204
Eurotiales	0.088574	0	0	0.021386	0.51013	0.0351	0.009132	0
Gloeophyllales	0	0	0	0.007129	0	0	0	0
Helotiales	0	0	0.022795	0.270887	0	0	0.027395	0
Hymenochaetales	0	0	0.091178	0.085543	0	0.0351	0.100447	0.010204
Hypocreales	0.620018	0	0	0	0.043725	0	0	0
Malasseziales	91.05403	99.93257	94.09619	92.76447	96.07929	98.3503	95.16939	98.46939
Ostropales	0	0	0	0	0	0	0.018263	0
Pezizales	0	0	0	0	0	0	0	0.010204
Pleosporales	0.177148	0	0.022795	0.270887	0	0	0.009132	0.05102
Pneumocystidales	0	0	0	0	0.014575	0	0	0
Polyporales	2.214349	0.029969	2.07431	1.11919	0.685031	0.526501	1.762396	0.683673
Pucciniales	0	0	0	0.021386	0	0	0	0

Russulales	0	0	0.934579	0.220987	0	0	0.538764	0.071429
Saccharomycetales	0.265722	0	0	0.035643	0.072876	0.0702	0	0.020408
Spizellomycetales	0	0	0	0.007129	0	0	0	0
Tremellales	4.428698	0	0.022795	0.149701	1.953068	0.0351	0.045658	0.010204
Wallemiales	0	0	0	0	0.043725	0.0351	0	0
Xylariales	0.265722	0	0	0.064157	0.02915	0.1053	0	0.010204

* Allergic rhinitis sample; ** Healthy control

Table S4. Relative proportion of fungal families

	A1*	A2	A3	A4	H1**	H2	H3	H4
Agaricaceae	0.08881	0	0	0	0	0	0	0
Agaricales incertae sedis	0.08881	0	0	0	0.072897	0	0	0
Agaricostilbaceae	0	0	0	0	0	0	0.018387	0
Ascomycota incertae sedis	0	0	0	0.021594	0	0	0	0
Atheliaceae	0	0	0	0	0.014579	0.035236	0.027581	0
Botryobasidiaceae	0	0	0	0.007198	0	0	0	0
Calosphaeriaceae	0	0	0	0.007198	0.029159	0	0	0
Capnodiales incertae sedis	0	0	0	0.014396	0	0.105708	0	0
Ceratobasidiaceae	0	0	0.068776	0.064781	0	0	0	0
Cordycipitaceae	0.62167	0	0	0	0.043738	0	0	0
Coriolaceae	2.220249	0.007493	0.985786	0.604621	0.670652	0.528541	0.81824	0.224972
Cryphonectriaceae	0	0	0	0.014396	0	0	0	0
Cystofilobasidiaceae	0	0	0	0	0.043738	0.035236	0	0
Davidiellaceae	0	0.007493	0.022925	0.151155	0	0	0.119518	0.061356
Dermateaceae	0	0	0	0.007198	0	0	0	0
Diatrypaceae	0.26643	0	0	0.043187	0.029159	0.105708	0	0.010226
Didymellaceae	0.08881	0	0	0.093572	0	0	0	0.040904
Dothioraceae	0	0	0	0.050385	0	0	0.045969	0
Exidiaceae	0	0	0.022925	0	0	0	0.009194	0
Gloeophyllaceae	0	0	0	0.007198	0	0	0	0
Gnomoniaceae	0	0	0.068776	1.065285	0	0	0.036775	0.05113
Herpotrichiellaceae	0	0	0.022925	0	0.014579	0	0	0
Hyaloscyphaceae	0	0	0	0.035989	0	0	0	0
Hymenochaetaceae	0	0	0.068776	0.064781	0	0.035236	0.101131	0.010226

Hymenochaetales incertae sedis	0	0	0	0.021594	0	0	0	0
Lachnocladiaceae	0	0	0.045851	0.007198	0	0	0	0.010226
Malasseziaceae	91.29663	99.94755	94.63549	93.66587	96.1073	98.7315	95.81686	98.68085
Marasmiaceae	0.26643	0	0	0	0	0	0.009194	0
Mycosphaerellaceae	0	0	0.091701	0.079177	0.014579	0	0	0.010226
Olpidiaceae	0	0	0	0.007198	0	0	0	0
Pezizaceae	0	0	0	0	0	0	0	0.010226
Phaeosphaeriaceae	0	0	0	0.021594	0	0	0	0
Phanerochaetaceae	0	0	0.022925	0	0	0	0.036775	0.010226
Pleosporaceae	0	0	0.022925	0	0	0	0	0
Pleosporales incertae sedis	0	0	0	0.021594	0	0	0	0
Pleurotaceae	0	0	0	0.007198	0	0	0	0
Pneumocystidaceae	0	0	0	0	0.014579	0	0	0
Polyporaceae	0	0	0.229253	0.035989	0	0	0.156293	0.010226
Polyporales incertae sedis	0	0.007493	0.298028	0.179947	0	0	0.257424	0.235198
Psathyrellaceae	0	0	0	0.007198	0	0	0.009194	0
Pucciniaceae	0	0	0	0.021594	0	0	0	0
Saccharomycetaceae	0.08881	0	0	0.021594	0.072897	0.070472	0	0.020452
Saccharomycodaceae	0	0	0	0.014396	0	0	0	0
Schizophyllaceae	0.08881	0.029974	2.361302	3.347009	0.116635	0.035236	1.939873	0.521526
Schizoporaceae	0	0	0.022925	0	0	0	0	0
Stereaceae	0	0	0.917011	0.20154	0	0	0.542429	0.071582
Stictidaceae	0	0	0	0	0	0	0.018387	0
Tremellaceae	4.440497	0	0.022925	0.050385	1.953638	0.035236	0.036775	0.010226
Trichocomaceae	0.08881	0	0	0.021594	0.510278	0.035236	0	0
Tricholomataceae	0	0	0.045851	0	0.131214	0	0	0

Tulasnellaceae	0	0	0	0	0	0	0	0.010226
Valsaceae	0.35524	0	0	0.014396	0.116635	0.211416	0	0
Wallemiaceae	0	0	0	0	0.043738	0.035236	0	0

* Allergic rhinitis sample; ** Healthy control

Table S5. Relative proportion of fungal genera

	A1*	A2	A3	A4	H1**	H2	H3	H4
<i>Agaricus</i>	0.089526	0	0	0	0	0	0	0
<i>Alternaria</i>	0	0	0.023089	0	0	0	0	0
<i>Antrodia</i>	0	0	0.023089	0.050659	0	0	0.036921	0
<i>Antrodiella</i>	0	0	0.023089	0	0.073378	0	0	0
<i>Aspergillus</i>	0	0	0	0.014474	0	0	0	0
<i>Bensingtonia</i>	0	0	0	0	0	0	0.01846	0
<i>Bjerkandera</i>	0	0	0.023089	0.043422	0	0	0.092302	0.04095
<i>Botryobasidium</i>	0	0	0	0.007237	0	0	0	0
<i>Catenulostroma</i>	0	0	0	0	0	0.105746	0	0
<i>Ceriporiopsis</i>	0.358102	0	0	0	0	0	0.027691	0
<i>Cerrena</i>	0	0	0.023089	0.007237	0.029351	0	0.027691	0.020475
<i>Chromocleista</i>	0	0	0	0.007237	0	0	0	0
<i>Cladosporium</i>	0	0	0	0.021711	0	0	0.01846	0.030713
<i>Columnosphaeria</i>	0	0	0	0.014474	0	0	0	0
<i>Coprinellus</i>	0	0	0	0.007237	0	0	0.00923	0
<i>Cryphonectria</i>	0	0	0	0.014474	0	0	0	0
<i>Cryptococcus</i>	4.476276	0	0.023089	0.043422	1.966539	0.035249	0.027691	0.010238
<i>Daedaleopsis</i>	0.80573	0	0.484876	0.275004	0.308189	0.317237	0.378438	0.0819
<i>Davidiella</i>	0	0.007493	0	0.050659	0	0	0.064611	0.010238
<i>Debaryomyces</i>	0	0	0	0	0	0.070497	0	0
<i>Diatrype</i>	0.268577	0	0	0	0.029351	0.070497	0	0
<i>Dothiora</i>	0	0	0	0	0	0	0.046151	0
<i>Fibulorhizoctonia</i>	0	0	0	0	0.014676	0.035249	0	0

<i>Porostereum</i>	0	0	0.023089	0	0	0	0.036921	0.010238
<i>Powellomyces</i>	0	0	0	0.007237	0	0	0	0
<i>Pseudorobillarda</i>	0	0	0	0.021711	0	0	0	0
<i>Puccinia</i>	0	0	0	0.021711	0	0	0	0
<i>Pycnoporus</i>	0	0	0	0.028948	0	0	0	0
<i>Ramichloridium</i>	0	0	0	0.014474	0	0	0	0
<i>Resinicium</i>	0	0	0	0.021711	0	0	0	0
<i>Saccharomyces</i>	0.089526	0	0	0.007237	0.073378	0	0	0
<i>Schizophyllum</i>	0.089526	0.029974	2.355114	3.365176	0.117405	0.035249	1.947572	0.522113
<i>Septoria</i>	0	0	0.092357	0.072369	0	0	0	0.010238
<i>Stereum</i>	0	0	0.092357	0.036185	0	0	0.119993	0.030713
<i>Strobilurus</i>	0	0	0	0	0.029351	0	0	0
<i>Togninia</i>	0	0	0	0.007237	0.014676	0	0	0
<i>Trametes</i>	1.074306	0.007493	0.323251	0.16645	0.264162	0.211491	0.249215	0.0819
<i>Trichaptum</i>	0	0	0.230894	0.007237	0	0	0.138453	0.010238
<i>Tulasnella</i>	0	0	0	0	0	0	0	0.010238
<i>Tyromyces</i>	0	0	0	0.036185	0	0	0.00923	0
<i>Udeniomyces</i>	0	0	0	0	0.044027	0.035249	0	0
<i>Valsa</i>	0.358102	0	0	0	0.10273	0.211491	0	0
<i>Wallemia</i>	0	0	0	0	0.044027	0.035249	0	0
<i>Zasmidium</i>	0	0	0	0	0.014676	0	0	0

* Allergic rhinitis sample; ** Healthy control

Table S6. Relative proportion of *Malassezia* species

	A1*	A2	A3	A4	H1**	H2	H3	H4
<i>M. cuniculi</i>	0	0	0	0.007685	0	0	0	0
<i>M. dermatis</i>	3.501946	0	0.121124	1.01437	0.03034	0.035689	0	0.031088
<i>M. globosa</i>	1.653696	0.074974	0.193798	1.314071	0.743325	0.035689	0.009595	0.559585
<i>M. obtusa</i>	0	0	0	0.015369	0	0	0	0
<i>M. pachydermatis</i>	0.097276	0.02999	0.65407	1.022055	0	0.071378	0.307043	0.632124
<i>M. restricta</i>	86.4786	99.33273	98.88566	96.08084	98.95328	99.75018	99.42429	98.42487
<i>M. slooffiae</i>	8.07393	0.007497	0.121124	0.230539	0.242718	0.035689	0.03838	0.062176
<i>M. sympodialis</i>	0.097276	0	0	0.092215	0	0.071378	0	0.020725
unclassified	0.097276	0.554806	0.024225	0.222854	0.03034	0	0.220687	0.26943

* Allergic rhinitis sample; ** Healthy control