Experimental Dermatology

WILEY

Experimental Dermatology

RESEARCH ARTICLE OPEN ACCESS

The Skin Mycobiome of Patients With Atopic Dermatitis and Healthy Volunteers: A Case–Control Study

Magdalena Żychowska[1] | Zofia Bakuła[2] | Przemysław Decewicz[3] | Anita Hryncewicz-Gwóźdź[4] | Mariusz Dyląg[2] | Alina Jankowska-Konsur[5] | Jan Gawor[6] | Robert Gromadka[6] | Anna Żaczek[7] | Tomasz Jagielski[2]

[1]Department of Dermatology, Faculty of Medicine, Collegium Medicum, University of Rzeszow, Rzeszow, Poland | [2]Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland | [3]Department of Environmental Microbiology and Biotechnology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland | [4]Department of Dermatology, Venereology and Allergology, Faculty of Medicine and Dentistry, Wroclaw Medical University, Wroclaw, Poland | [5]Faculty of Medicine and Dentistry, University Centre of General Dermatology and Oncodermatology, Wroclaw Medical University, Wroclaw, Poland | [6]DNA Sequencing and Oligonucleotide Synthesis Laboratory, Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland | [7]Department of Microbiology, Faculty of Medicine, Collegium Medicum, University of Rzeszow, Rzeszow, Poland

Correspondence: Tomasz Jagielski (t.jagielski@uw.edu.pl)

Received: 19 August 2024 | **Revised:** 7 February 2025 | **Accepted:** 8 March 2025

Funding: This work was supported by the Iuventus Plus grant from the Polish Ministry of Science and Higher Education (0258/IP1/2016/74).

Keywords: atopic dermatitis | dysbiosis | fungi | ITS | metataxonomy | mycobiome | sequencing | skin

ABSTRACT

Atopic dermatitis (AD) is a common inflammatory skin disease, for which dysbiosis of the skin mycobiome is considered a triggering factor. The aim of this study was to explore the skin mycobiome of AD patients and healthy volunteers (HV). The study included 50 AD patients and as many HV. Culture-based species identification involved a battery of conventional phenotypic tests and PCR sequencing of the internal transcribed spacer (ITS) 1 and 2 regions within the rDNA cluster. Culture-independent, metataxonomic sequencing was performed with ITS1 as the target region. The overall culture-positive rate was higher in AD patients than in HV (74% vs 28%). Among the former, *Rhodotorula* spp. dominated, followed by *Candida* spp., *Malassezia* spp. and *Naganishia albida*. The congruence between PCR sequencing and phenotyping was 68.6%. Upon metataxonomy of AD samples, 33 (66%) demonstrated close clustering with HV samples ('control-like' AD), while 17 (34%) displayed a remarkably different mycobiome composition ('AD-specific'), with *Cladosporium*, *Malassezia*, *Candida*, *Diplodia*, *Saccharomyces*, *Penicillium* and *Aspergillus* genera showing increased abundance. Patients with 'AD-specific' mycobiomes were more commonly exposed to air-conditioning compared to 'control-like' AD patients ($p = 0.030$). A subset of patients with AD has a different cutaneous mycobiome make-up dominated by environmental moulds, and *Malassezia* and *Candida* yeasts. Anthropogenic factors may affect the cutaneous mycobiome composition in AD and should be taken into account in microbiome studies.

1 | Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin condition affecting up to 20.1% of children and 4.9% of adults worldwide [1, 2]. A number of factors, including genetic background, abnormal epidermal barrier, disturbed immune responses and dysbiosis, are considered to be involved in the pathogenesis of the disease [3].

Fungi make up an important part of the skin microbiome, and as such may be implicated in the pathogenesis of various dermatological conditions [4, 5]. Impaired epidermal barrier in AD,

along with increased T-cell reactivity, may favour sensitisation to fungal allergens [4].

Until recently, data on the human mycobiome have largely relied on cultured microorganisms identified by culture-dependent methods. Studies using these methods have linked *Malassezia* and *Candida* spp. to AD flares and highlighted the role of these fungi in cutaneous sensitisation [5–7]. This has been further supported by the more frequent occurrence of IgE antibodies against *Malassezia* in AD patients in comparison with healthy, nonatopic individuals [6].

The development of advanced molecular techniques, and next-generation sequencing in particular, has enabled the investigation of microbial communities without the need for cultivation. For instance, reduced bacterial alpha-diversity, due to the predominance of *Staphylococcus aureus* in the skin microbiome, has been demonstrated in multiple metagenomic studies [8–10]. However, molecular-based data on the role of fungi and skin mycobiome composition in this dermatosis are still very limited [8, 11–13].

The aim of the study was to explore the occurrence and diversity of fungi on the skin of AD patients and healthy volunteers (HV) by using a two-pronged strategy, that is, involving culture-dependent and culture-independent approaches.

2 | Materials and Methods

2.1 | Patient Recruitment

Patients with AD, aged 15 years or older, who presented to the Department of Dermatology, Venereology and Allergology in Wroclaw (Poland) between January 2017 and May 2019 were recruited for the study. All patients fulfilled the Hanifin–Rajka diagnostic criteria for AD [14]. The exclusion criteria included concomitant dermatological (e.g., psoriasis, allergic contact dermatitis, lichen planus) or systemic conditions (e.g., asthma, food allergies, diabetes, polycystic ovary syndrome) that might have affected the skin mycobiome composition, the use of systemic or topical treatment for AD within the preceding 12 or 4 weeks, respectively, and the use of systemic or topical antibacterial and/or antifungal agents within the preceding period of 6 months.

Sociodemographic data, including sex, age, profession, presence of pets at home, exposure to moulds/humidity, having air-conditioning at home/work and going to the swimming pool. were collected by the investigators using a specially designed questionnaire (Appendix S1). The impact of AD on the patient's quality of life was evaluated using the Dermatology Life Quality Index (DLQI) [15]. The intensity of pruritus was measured using the visual analogue scale (VAS) [16].

The severity of AD was assessed using Severity Scoring of Atopic Dermatitis (SCORAD) [17]. For every AD patient, the laboratory parameters (IgE level, C-reactive protein concentration and presence of eosinophilia) were measured.

The control group consisted of HV ≥ 18 years of age, with a negative history for atopy or AD, who presented for a routine nevi check-up.

This work was approved by the local Ethics Committee of the Wroclaw Medical University (Decision no. 23/2016) and authors can confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The study was conducted according to the Declaration of Helsinki. All study participants signed the written informed consent form prior to any procedure. In the case of juvenile patients (< 18 years), the consent was signed by parents or legal guardians.

2.2 | Sample Collection

In AD, skin samples for culture-dependent species identification were collected from three locations, that is, antecubital crease, neck and popliteal fossa. Samples for the metataxonomic analysis were taken from active lesions, preferentially located in the antecubital creases ($n = 44$), or the neck ($n = 6$). Prior to sampling, the designated skin areas were left untreated for at least 4 weeks. All participants were instructed to avoid washing for 24 h before material collection.

In the control group, samples from three locations (antecubital crease, neck, popliteal fossa) were taken for culture-dependent speciation, and a single sample from healthy skin in the antecubital crease was collected for metataxonomic analysis.

All procedures were performed using sterile gloves. In atopic dermatitis patients, the typically rough skin surface was scraped with a sterile disposable scalpel (Swann Morton, Sheffield, UK), and the scrapings were placed in a sterile Petri dish. In the control group, where it was impossible to collect skin scrapings with a scalpel without causing small cuts to the healthy skin, the samples were collected using OpSite dressings (Smith & Nephew Education, Watford, UK).

2.3 | Culture-Dependent Species Identification

2.3.1 | Conventional Mycological Diagnostics

Collected samples were inoculated aseptically onto yeast extract peptone dextrose agar (YPDA) and Modified Leeming and Notman agar (MLNA), prepared as described elsewhere [18, 19], supplemented with gentamicin (0.01%), chloramphenicol (0.005%) and cycloheximide (0.05%) [20, 21]. Plates were incubated at 28°C (YPDA) or at 32°C (MLNA) for up to 14 days. All isolates growing on MLNA and unable to grow on YPDA were diagnosed according to commonly known protocols (Appendix S2 and Figure 1 green panels). Differentiation and species identification of filamentous and yeast-like fungi were carried out following standard protocols and approved algorithms for dermatophytes and yeast-like fungi (Appendix S2 and Figure 1 orange and blue panels, respectively).

2.3.2 | PCR-Based Species Identification

Species-level identification was achieved by sequencing the ITS1 and ITS2 regions within the rDNA cluster, with ITS4 and ITS5 primers [12]. (Appendix S3).

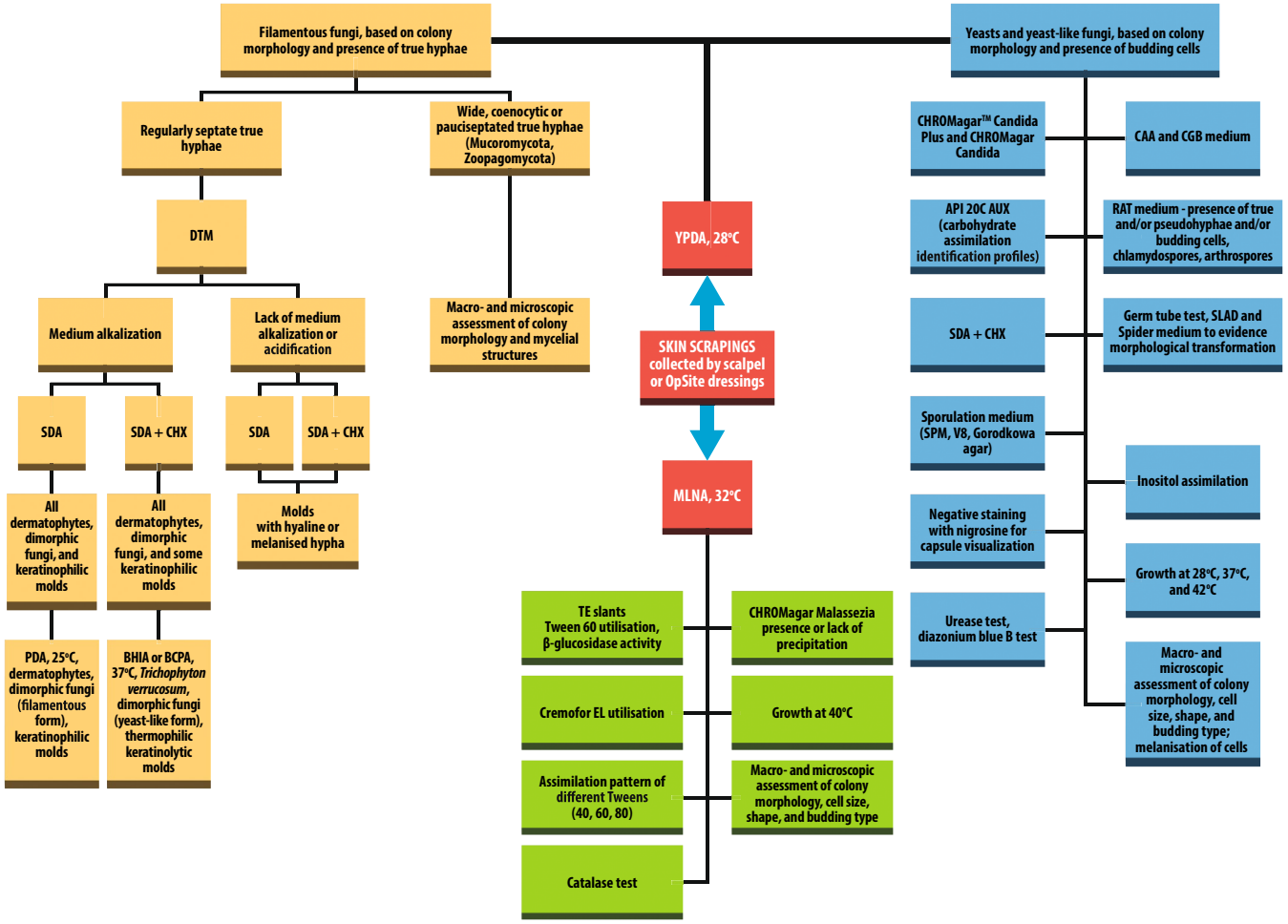

FIGURE 1 | Conventional, phenotype-based species identification algorithm applied in this study. API 20C AUX, name of commercially available test; BCPA, brom cresol purple agar; BHIA, brain heart infusion agar; CAA, caffeic acid agar; CGB, canavanine-glycine-bromothymol blue; CHX, cycloheximide; DTM, dermatophyte test medium; MLNA, modified Leeming & Notman agar; PDA, potato dextrose agar; RAT, rice agar Tween; SDA, Sabouraud dextrose agar; SLAD, synthetic low ammonium dextrose; SPM, sporulation medium; TE, tween esculin; V8, ATCC Medium 343: V-8 juice agar; YPDA, yeast extract peptone dextrose agar. For detailed information and composition of used culture media please see Appendix S2 and cited herein literature.

The sequences determined were deposited in the GenBank database under the accession numbers provided in Table S1.

2.4 | Culture-Independent Species Identification

2.4.1 | DNA Isolation

DNA for metataxonomic analysis was isolated using the GeneMATRIX Environmental DNA & RNA Purification Kit (EURx, Gdansk, Poland), as per the manufacturer's instructions. Purified DNA, dissolved in TE buffer, was quantified with the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and stored at −20°C until use.

2.4.2 | Metataxonomic Sequencing

PCR amplicons for metataxonomic analyses were based on the ITS1 fungal marker region. Primers ITS1 5′-TCCGTAGGTGAAC CTGCGG-3′ and ITS2 5′-GCTGCGTTCTTCATCGATG-3′ were extended with Illumina adapters and used for the PCR reaction [22, 23]. Obtained amplicons (ca. 300 bp) were checked on a 1% agarose gel and purified by Ampure XP magnetic beads (Beckman Coulter, Brea, USA). Amplicon libraries were pooled in an equimolar ratio and indexed according to the Nextera indexing strategy by PCR (Illumina, San Diego, USA). Sample indexing allowed pooling of amplicons for the sequencing run and further extraction of the sample sequence reads from a large batch of sequencing results data.

To minimise technical bias, three technical replicates were prepared and pooled for each PCR amplicon. Amplicon libraries were prepared using MiSeq Reagent Kit v3 and sequenced on an Illumina MiSeq instrument (Illumina, San Diego, USA) in the DNA Sequencing and Oligonucleotide Synthesis Laboratory—oligo.pl. IBB PAS in a paired-end mode (2×300 cycles). The sequencing resulted in the acquisition of 31 919 886 pairs of raw reads in total (from 96 448 to 1 130 136 read pairs per sample) (Appendix S3 and S4).

2.5 | Statistical Analysis

Calculations were performed using Statistica 13.0. Categorical variables were presented as frequencies with percentages, and continuous variables were reported as medians with ranges. Fisher's exact test was used for comparisons of categorical variables, and the Wilcoxon rank test was used for continuous variables. Statistical differences between alpha- and beta-diversity were calculated using the Kruskal–Wallis test with Bonferroni correction and ANOSIM test with 999 permutations, respectively. Differentially abundant ASVs and taxonomic groups were determined based on ANCOM analyses using the QIIME2 composition plugin. A p value < 0.05 was considered statistically significant.

3 | Results

3.1 | Study Population

50 AD patients (mean age ± standard deviation (SD) 31.8 ± 13.15 years; 24 females and 26 males) and 50 HV (mean age 37.7 ± 14.28 years; 39 females and 11 males) were included. Both in AD and control groups, the majority of subjects were living in the urban area (78% vs 96%), were not exposed to moulds or humidity (88% vs 94%) and did not go to the swimming pool regularly (90% vs 88%). A substantial percentage of individuals had pets at home (40% of AD patients and 52% of HV) and were exposed to air-conditioning at home or work (38% of AD patients and 32% of HV; Table 1).

3.2 | Culture-Dependent Species Identification

The results of culture-dependent identification indicated a significantly higher number of fungi in AD patients compared to HV (Table 2). The overall culture-positive rate was 74% (37/50) for AD patients and 28% (14/50) for HV. Taking both groups together, a clear predominance of *Rhodotorula* spp. was observed among the isolates. Members of this genus were recovered at the highest frequency (21.6%), followed by *Candida* spp. (17.6%), *Malassezia* spp. (11.8%), and *Naganishia* spp. (7.8%). The percentage shares of *Rhodotorula*, *Candida, Malassezia* and *Naganishia* species among AD patients exclusively were 29.7%, 13.5%, 13.5% and 10.8%, respectively. Among HV, there were no strains of the *Rhodotorula* and *Naganishia* genera. Moreover, strains of the *Candida* and *Malassezia* genera accounted for either a higher or lower percentage of isolates compared with AD patients (28.6% and 7.1%, respectively).

Compared with conventional phenotyping, PCR-sequencing gave fully concordant identification results for 68.6% (35/51) of the strains cultured. For 5 (9.8%) strains, the two methods enabled a consistent genus assignment, but only genotyping permitted identification at the species level. For 11 (11/51; 21.6%) strains, the observed discrepancy concerned either genus (8/51; 15.7%) or species (3/51; 5.9%) level (Table 2).

3.3 | Culture-Independent Species Identification

3.3.1 | The Mycobiome of AD Patients Versus HV

First, the diversity and composition of the skin mycobiome in AD patients ($n = 50$) was compared to that of HV ($n = 50$). Chao1, Shannon's Index and Simpson's Index did not show any statistically significant differences in the fungal alpha-diversity between the two groups ($p = 0.879$, $p = 0.241$ and $p = 0.162$, respectively). However, statistical significance was reached for the Simpson evenness ($p < 0.001$). The PCoA visualisation of Bray–Curtis dissimilarity showed a high and statistically significant difference (ANOSIM, $p = 0.001$) in the fungal beta-diversity (inter-sample diversity) between the AD group and HV (Figure 2). Samples from 33 (66%) AD patients clustered closely with samples from HV, while the remaining samples from 17 (34%) AD patients were very scattered and remotely located away from the HV samples. Consequently, two groups of patients could be clearly distinguished: one, referred to as 'AD-specific' where mycobiomes differed importantly from those of HV, and the other, referred to as 'control-like' with mycobiomes essentially resembling those of HV.

3.3.2 | Clinical Characteristics of AD Patients With 'AD-Specific' Mycobiome

The sociodemographic and clinical parameters of AD patients with 'AD-specific' mycobiome were compared with those of 'control-like' AD patients and HV (Table 1). No significant differences were found between patients with 'AD-specific' mycobiome and 'control-like' AD patients in terms of gender, age, duration of the lesions, severity of AD, or laboratory parameters. Interestingly, 'AD-specific' mycobiome patients were significantly more frequently exposed to air-conditioning, either at home or at work ($p = 0.030$) when compared to 'control-like' AD patients. However, the difference did not reach statistical significance in a comparison with HV. Comparison between the three groups also showed higher percentages of participants living in urban areas and washing themselves regularly every day in the HV group ($p = 0.022$ and $p = 0.045$, respectively).

3.3.3 | The 'AD-Specific' Mycobiome

Patients with 'AD-specific' mycobiome ($n = 17$) were compared to 'control-like' AD patients ($n = 33$) and HV ($n = 50$). Chao1, Shannon's Index, Simpson's Index and Simpson's Evenness Index showed statistically significant differences in the fungal alpha-diversity between AD patients with 'AD-specific' mycobiome and 'control-like' AD patients ($p < 0.001$, $p < 0.0001$, $p < 0.0001$ and $p < 0.0001$ respectively). Similarly, statistically significant differences were observed for all alpha-diversity metrics in the comparison between the three groups ($p < 0.01$, $p < 0.0001$, $p < 0.0001$ and $p < 0.0001$ respectively; Figure 3).

ANCOM analysis showed a clear predominance of Ascomycota in 'AD-specific' mycobiomes while Basidiomycota outweighed among HV and 'control-like' AD patients.

TABLE 1 | Clinical characteristics of study participants.

Characteristics	AD				Healthy volunteers ($n=50$)	p^{**}
	Total ($n=50$)	Specific AD ($n=17$)	Control-like AD ($n=33$)	p^{*}		
Sex, *n* (%)						
Female	24	8	16	0.924	39	0.004
Male	26	9	17		11	
Age, years						
Range	15–65	16–53	15–65	0.652	19–68	0.049
Mean ± SD	31.8 ± 13.15	29.8 ± 10.8	32.8 ± 14.3		37.7 ± 14.28	
Median	29	29	29		32.5	
Living place, *n* (%)						
Urban area	39 (78.0%)	14	25	0.863	48	0.022
Rural area	11	3	8		2	
Farmer, *n* (%)						
Yes	2	0	2	0.784	0	0.126
No	48	17	31		50	
Pets at home, *n* (%)						
Yes	20	7	13	0.903	26	0.481
No	30	10	20		24	
Gardening, *n* (%)						
Yes	14	5	9	0.873	15	0.964
No	36	12	24		35	
Exposure to moulds/humidity, *n* (%)						
Yes	6	0	6		3	
No	44	17	27	0.157	47	0.06
Air-conditioning at home/work, *n* (%)						
Yes	19	10	9		16	
No	31	7	24	0.030*	34	0.07
Going to the swimming pool, *n* (%)						
Yes	5	2	3		6	
No	45	15	30	0.842	44	0.912
Frequency of body washing, *n* (%)						
Once a day	40	14	26	0.941	48	0.045
Less than once a day	10	3	7		2	

(Continues)

TABLE 1 | (Continued)

Characteristics	AD				Healthy volunteers ($n=50$)	p^{**}
	Total ($n=50$)	Specific AD ($n=17$)	Control-like AD ($n=33$)	p^{*}		
Age at first symptoms of ad, years						
Range	0.1–35	0.25–25	0.1–35		—	
Mean ± SD	6.6 ± 9.4	6.4 ± 9.2	6.8 ± 9.1	0.319	—	
Median	10.5	0.25	2		—	
Duration of current exacerbation, months						
Range	0.25–396	0.5–96	0.25–396		—	
Mean ± SD	39.2 ± 79.6	21.8 ± 26.6	49 ± 88.3	0.626	—	
Median	19	12	12		—	
Milk protein intolerance during infancy, *n* (%)						
Yes	24	10	14	0.272	—	
No	26	7	19		—	
Personal history of atopic-like symptoms other that ad, *n* (%)						
Yes	36	11	25	0.41	—	
No	14	6	8		—	
Family history of atopy, *n* (%)						
Yes	28	10	18	0.773	—	
No	22	7	15		—	
Prior topical treatment at sampling site, *n* (%)						
Corticosteroids	33	11	22	0.89	—	
None	17	6	11		—	
Prior systemic therapy, *n* (%)						
Immunosuppression (CCS/CsA/MTX/A)	21	6	15		—	
Phototherapy	4	4	1	0.06	—	
Immunosuppression + phototherapy	3	2	1		—	
None	16	5	16		—	
Total IgE				0.142		
Range	25–141 000	25–16 200	25–141 000		—	
Mean ± SD	12 058.1 ± 27 971.1	2298.7 ± 4079.7	22 656.1 ± 32 498.2		—	
Median	4945	978	7090		—	

(Continues)

TABLE 1 | (Continued)

Characteristics	AD				Healthy volunteers	
	Total ($n=50$)	Specific AD ($n=17$)	Control-like AD ($n=33$)	p*	($n=50$)	p**
C-reactive protein				0.94		
Normal (≤5)	31	12	19		—	
Above normal (>5)	7	2	5		—	
No data	12	3	9		—	
Eosinophilia				0.774		
Normal (0–0.6)	26	10	16		—	
Above normal (>0.6)	13	4	9		—	
No data	11	3	8		—	
SCORAD, points				0.73		
Range	10–75	21–72	10–75		—	
Mean ± SD	50 ± 14.6	45.6 ± 14.6	47.7 ± 14.8		—	
Median	46.5	48	46		—	
DLQI, points				0.792		
Range	1–35	4–27	1–35		—	
Mean ± SD	14.1 ± 7.8	12 ± 6.4	17.6 ± 7.8		—	
Median	17.5	12	19		—	
VAS				0.612		
Range	0–10	1–10	0–10		—	
Mean ± SD	5.6 ± 2.9	5.2 ± 2.7	5.9 ± 3		—	
Median	6	6	5.5		—	

Abbreviations: AD, atopic dermatitis; AZA, azathioprine; CCS, corticosteroids; CsA, cyclosporin A; MTX, methotrexate; SD, standard deviation.
*AD patients with 'AD-specific' mycobiome vs. 'control-like AD' mycobiome patients.
**AD patients with 'AD-specific' mycobiome vs. 'control-like AD' vs. control mycobiome patients.

At the family level, Trichosporonaceae dominated in HV and 'control-like' AD patients, while Cladosporiaceae, Saccharomycetales familiae incertae sedis and Malasseziaceae were most abundant in 'AD-specific' mycobiome. Cladosporiaceae, Malasseziaceae, Debaryomycetaceae, Saccharomycetales familiae incertae sedis, Botryosphaeriaceae, Saccharomycetales unassigned family, Aspergillaceae, Saccharomycetaceae and Sporidiobolaceae were significantly more abundant, whereas Pleosporales unassigned family, Thermoascaceae, Trichosporonaceae and Hypocreaceae were significantly underrepresented among 'AD-specific' mycobiome.

Finally, at the genus level, *Haglerozyma* was most abundant in HV and 'control-like' AD patients, while *Cladosporium*, *Candida* and *Malassezia* dominated in 'AD-specific' mycobiome. *Cladosporium*, *Debaryomycetaceae* unassigned genus, *Malassezia*, *Saccharomycetales* unassigned family unassigned genus, *Candida, Diplodia*, *Saccharomyces*, *Penicillium* and *Aspergillus* were significantly increased in 'AD-specific' mycobiome. Whereas *Haglerozyma*, *Byssochlamys*, *Trichoderma* and *Pleosporales* unassigned family unassigned genus were significantly more abundant in 'control-like' AD patients and HV (Figure 4).

4 | Discussion

The literature on the skin mycobiome in AD has been limited [4, 5, 11, 12, 24–33]. Studies exploiting culture-dependent identification methods have largely focused on yeasts, predominantly Malasseziomycetes [24–26]. These fungi were observed less frequently in AD patients than in HV or patients with seborrheic dermatitis [26].

High-throughput sequencing techniques allowed a much closer insight into the diversity of the skin mycobiome. Consequently, some important differences in the cutaneous mycobiome in AD patients and HV have been reported [4, 5, 11, 12, 30, 31, 34–36]. For instance, an increase in allergenic and/or pathogenic fungi, including *Alternaria*, *Fusarium* and *Scleroderma*, in the skin of AD patients with a positive atopic background was noted [36].

TABLE 2 | Species identification[a] using culture-dependent methods.

Group	Phenotyping	PCR sequencing	% Similarity[b]	No. of isolates	Location
AD	*Candida albicans*	*Candida albicans*	99.8%–100%	3	Neck ($n=1$) Popliteal fossa ($n=2$)
	Meyerozyma guilliermondii	*Meyerozyma guilliermondii*	100%	3	Popliteal fossa ($n=2$) Antecubital crease ($n=1$)
	Pichia kudriavzevii	*Pichia kudriavzevii*	100%	3	Neck ($n=2$) Antecubital crease ($n=1$)
	Yarrowia lipolytica	*Yarrowia lipolytica*	100%	1	Neck
	Clavispora lusitaniae	*Clavispora lusitaniae*	99.7%	1	Popliteal fossa
	Candida parapsilosis	*Candida parapsilosis*	100%	1	Neck
	Candida sp.	*Candida zeylanoides*	100%	1	Popliteal fossa
	Filobasidium uniguttulatum	*Filobasidium uniguttulatum*	100%	1	Neck
	Malassezia globosa	*Malassezia globosa*	99.0%–99.3%	2	Neck ($n=2$)
	Malassezia sympodialis	*Malassezia sympodialis*	99.8%–100%	3	Neck ($n=2$) Antecubital crease ($n=1$)
	Naganishia albida[c]	*Naganishia diffluens*	99.70%–100%	4	Neck ($n=2$) Popliteal fossa ($n=1$) Antecubital crease ($n=1$)
	Rhodotorula mucilaginosa	*Rhodotorula mucilaginosa*	99.3%–100%	9	Neck ($n=5$) Popliteal fossa ($n=4$)
	Rhodotorula mucilaginosa	*Rhodotorula diobovata*	99.7%	1	Antecubital crease
	Rhodotorula sp.	*Rhodotorula diobovata*	100%	1	Antecubital crease
	Saccharomyces sp.	*Nakazawaea holstii*	98%	1	Neck
	Trichosporon beigelii	*Cutaneotrichosporon dermatis*	100%	1	Neck
	Wickerhamia fluorescens	*Wickerhamia fluorescens*	99.4%	1	Neck
Control	*Acremonium sp.*	*Phialemonium sp.*	100%	1	Popliteal fossa
	Candida albicans	*Candida albicans*	100%	3	Neck ($n=1$) Popliteal fossa ($n=1$) Antecubital crease ($n=1$)
	Pichia kudriavzevii	*Pichia kudriavzevii*	100%	1	Popliteal fossa
	Hanseniaspora sp.	*Candida tropicalis*	100%	1	Neck
	Malassezia sympodialis	*Malassezia sympodialis*	100%	1	Neck
	Ogataea methanolica	*Ogataea methanolica*	99.5%	1	Neck
	Saccharomyces cerevisiae	*Saccharomyces cerevisiae*	100%	1	Neck
	Sporobolomyces sp.	*Sporobolomyces roseus*	99.6%	1	Popliteal fossa
	Sterigmatomyces sp.	*Cutaneotrichosporon curvatum*	100%	1	Antecubital crease
	Cutaneotrichosporon cutaneum	*Apiotrichum domesticum*	99.8%	1	Popliteal fossa

(Continues)

TABLE 2 | (Continued)

Group	Phenotyping	PCR sequencing	% Similarity[b]	No. of isolates	Location
	Cutaneotrichosporon mucoides	*Apiotrichum montevideense*	100%	1	Neck
	Zygosaccharomyces rouxii	*Saccharomyces cerevisiae*	99.6%	1	Neck
Total				51	

[a]Nomenclature according to MYCOBANK Database and Taxonomy Browser. Available online at https://www.mycobank.org/ and Taxonomy browser (root) (nih.gov) accessed on: August 9th 2024.
[b]First hit according to BLAST Refseq ITS database (https://www.ncbi.nlm.nih.gov/refseq/).
[c]*Naganishia albida*, formerly known as *Cryptococcus albidus*.

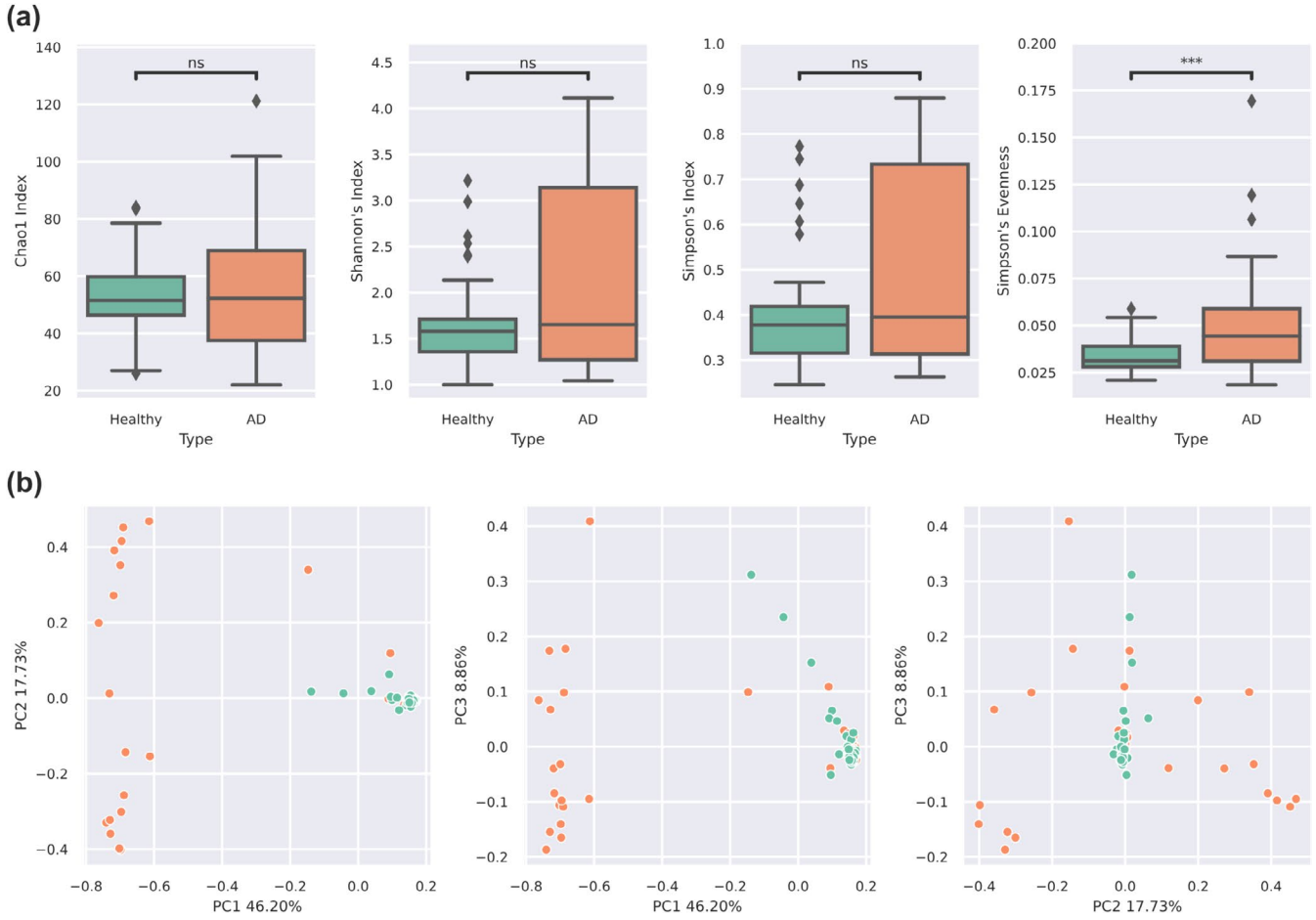

FIGURE 2 | Alpha- and beta-diversity analyses of examined fungal communities in the case of AD patients and healthy volunteers. On panel (a) alpha-diversity of selected metrics was presented with the results of statistical inference. The 'ns' abbreviation was used for 'not significant', while *** indicates the *p* value of the Kruskal–Wallis test with Bonferroni correction lower than 0.001. On panel (b) PCoA plot based on Bray–Curtis dissimilarity was shown to visualise the intersample diversity between atopic dermatitis (AD) and healthy individuals. On each panel, a combination of the first three principal components was shown with the level of dissimilarity they represent. The sample category colouring scheme between panels is the same.

In this work, conventional identification methods produced a higher culture-positive rate in AD patients than in HV. Moreover, culture-based phenotypic and PCR-sequencing methods used for species identification gave concordant results for 68.6% of the isolates. Such discrepancies only confirm the superiority of molecular methods over conventional, culture-based mycological diagnostics [25].

The mycobiome composition, as assessed by culture-dependent and independent methods, was only partially overlapping. The discrepancies may result from different growth or metabolic capacities of fungi. Methods of metataxonomy disclose higher species richness and overall biodiversity, whereas culture-based tests reflect more accurately the potential of individual species to proliferate in given conditions [4, 37].

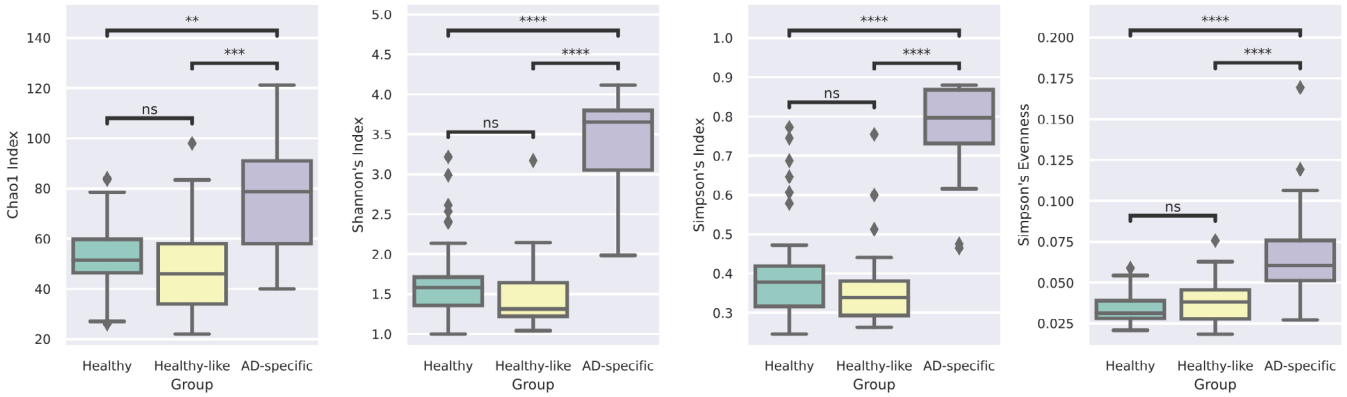

FIGURE 3 | Alpha-diversity analyses of examined fungal communities in the case of AD-specific, healthy and healthy-like groups. The alpha-diversity of selected metrics was presented with the results of statistical inference. The marks **, *** and **** indicate the *p* values of the Kruskal–Wallis test with Bonferroni correction lower than 0.01, 0.001, and 0.0001, respectively. The ns indicates no significant differences between groups.

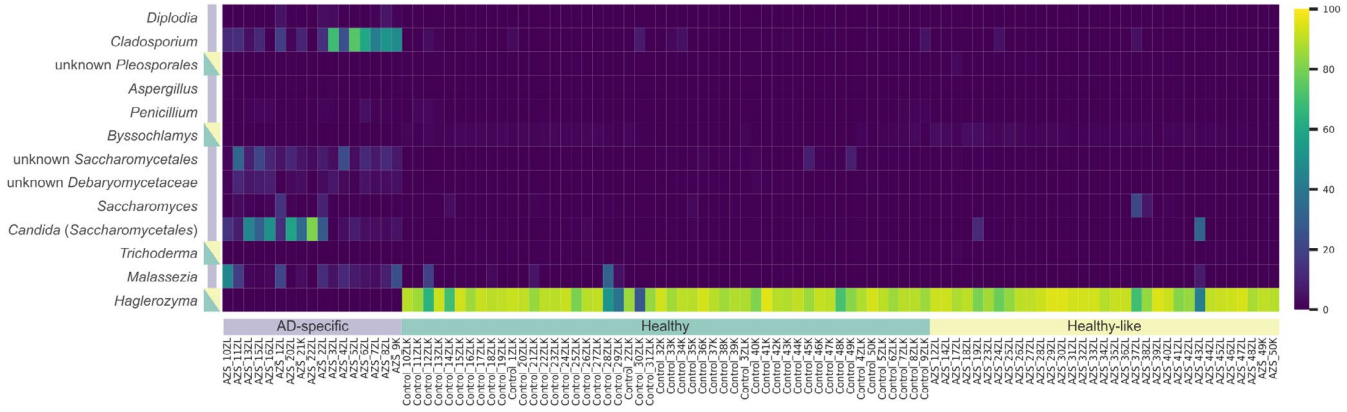

FIGURE 4 | Heatmap showing genera with significantly different abundance between atopic dermatitis (AD-specific), healthy and healthy-like groups of volunteers. The scale bar's colours reflect their relative abundance. Coloured blocks near taxa names indicate the group of samples that were indicated to be significantly more abundant in the ANCOM analysis.

Conventional analysis indicated a significantly greater share of Basidiomycota (especially basidiomycetous yeasts) in the skin mycobiome of AD patients, while Ascomycota, especially ascomycetous yeasts, dominated among HV. This is exactly the opposite of what was found using metataxonomy.

Both methods revealed a clear predominance of opportunistic fungal pathogens in AD patients compared to HV. However, whereas *Rhodotorula*, *Candida, Malassezia* and *Trichosporon* fungi dominated in conventional analysis, *Cladosporium*, *Malassezia*, *Candida, Penicillium* and *Aspergillus* were in the majority upon metataxonomic analysis.

Similarities in the abundance of *Candida* and *Malassezia* in the AD mycobiome confirm the ability of both culture-dependent and culture-independent methods to identify the dominant taxa. *Malassezia* is a lipophilic yeast, whose role in the pathogenesis of skin inflammation may stem from its lipases and phospholipases. The enzymes damage the epidermis, trigger an immune reaction and provoke the production of inflammatory cytokines [38]. Sensitisation to *Malassezia* spp. has been demonstrated to constitute an important AD severity marker [39]. Prior culture-independent studies, implementing sequencing methods, found a high prevalence of *Malassezia* spp. in AD samples, with two species, *M. slooffiae* and *M. dermatitis*, being specific for AD [5]. However, the study was carried out on a very small group of 8 AD patients and 8 HV. The higher prevalence of *Malassezia* spp., especially *M. globosa* and *M. restricta*, in AD patients than in healthy subjects was also found by Edslev et al. [12], yet the difference was not statistically significant. In another study, *Malassezia* was again found to be the most abundant fungal genus. Importantly, the authors observed an increase in allergenic and/or pathogenic fungi, including *Alternaria*, *Fusarium* and *Scleroderma*, in the skin of AD patients with a positive atopic background [36].

Candida is another commonly present yeast on human skin, which may stimulate immediate hypersensitivity [40]. The results of the culture-based studies analysing the carriage of *Candida* spp. in patients with AD have been contradictory [27–29]. Frequent *Candida* spp. colonisation in patients with AD was reported by Arzumanyan et al. [27], while these results were not confirmed by Javad et al. [28] In a recent metataxonomic study, Schmid et al. [11] analysed the skin swabs of 16 patients with AD by sequencing the ITS1 region and found increased abundance of *Candida* spp. over *Malassezia* spp. in severe AD. These findings are also consistent with the results reported by Storz et al. [40], who, along with abundant colonisation with *Candida* in AD, found increased sensitisation towards

C. albicans. It should be taken into consideration that individual studies were conducted on different patient populations, living in different conditions and leading different lifestyles, which could have had an impact on the composition of the mycobiomes and explain the differences in the results.

The most pronounced difference between the culture-dependent and culture-independent identification methods concerned environmental moulds (*Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp.), which were highly abundant in the metataxonomic analysis, albeit virtually undetected with culture-dependent methods. This might be surprising, since environmental moulds are frequently identified in culture due to their fast proliferation and efficient conidia production [37]. These species are typical components of bioaerosols of indoor and outdoor air and are well-known air-conditioner colonisers [41]. A compelling explanation relates to the inability of the metataxonomic approach to unequivocally distinguish between environmentally derived and factually colonising microorganisms [27, 33].

Moreover, metataxonomy allows the detection of fungal species irrespective of their metabolic activity, including poorly growing and nonviable fungal cells [27, 33]. This may explain the presence of many fungal species, especially moulds, which remain undetected with culture-dependent methods.

So far, few studies have looked at moulds in the cutaneous mycobiome in AD. In one study [8], an increased abundance of *Cladosporium* was correlated with transepidermal water loss (TEWL), as well as with staphylococcal colonisation. In another study, an increased abundance of *Alternaria* in AD patients was demonstrated, along with biofilm formation, composed of *Staphylococcus* and *Alternaria*, stimulating the production of proinflammatory cytokines by keratinocytes [8, 36]. In AD, epidermal barrier impairment may favour the penetration of environmental moulds into the epidermis and subsequent development of Th2 immune response and atopic manifestations.

Interestingly, we observed significantly more frequent exposure to air-conditioning either at home or at work in patients with 'AD-specific' mycobiome than in the 'control-like' AD patients. Dry air in air-conditioned rooms is known to exacerbate skin problems, with AD in the first place [28, 29, 41]. However, in this case, the link between exposure to air-conditioning and AD flares needs further elucidation.

Fungi, especially *Malassezia*, have also been linked to exacerbations of another chronic skin disease, psoriasis [42]. Choi et al. [43] found a correlation between increased mycobiome diversity and the severity of scalp psoriasis, with increased abundance of *Malassezia globosa* in the most severe cases. In addition, the treatment of psoriasis with anti-IL-23 antibodies was demonstrated to affect the cutaneous mycobiome by reducing its alpha-diversity and the relative abundance of the *Malassezia* genus [44]. The effectiveness of antifungal therapies in controlling the symptoms of chronic diseases such as AD or psoriasis [45] is a premise for further research on the role of the mycobiome in the development of cutaneous inflammation.

Although our results come from a single-centre study, the advantage of our work is a large sample size [5, 8]. So far, only one metataxonomic study on the skin mycobiome in AD included a comparable number of participants, namely 58 AD patients and 46 HV [12]. Another advantage is the analysis of factors that might have potentially influenced the composition of the mycobiome, such as swimming pool use, exposure to air-conditioning, or having pets at home. In addition, contrary to previous works [5, 8, 12], our study implemented a combination of culture-dependent species identification and metataxonomic analysis of the fungal profile in AD.

One of the limitations of the study, which requires further analysis in the future, is the lack of evaluation of sensitisation to individual identified fungi in AD patients enrolled for the study. Such an assessment would allow for a better understanding of the cause-and-effect relationship between the mycobiome composition and the occurrence of AD symptoms. Another limitation is the difference in the proportion of females and males between the AD group and HV. Although the gender-related differences in mycobiome composition are well known, we believe that this disproportion, given the large group of study participants, should not considerably impact the findings and final conclusions.

In conclusion, our study adds to the evidence that the cutaneous mycobiome in AD differs from that in the healthy population. Importantly, a subgroup of AD patients with a distinct mycobiome composition, dominated by moulds of the *Cladosporium*, *Aspergillus* and *Penicillium* genera, along with *Malassezia* and *Candida* yeasts, could be distinguished. It seems that anthropogenic factors, such as exposure to air-conditioning, may have a role in shaping the mycobiome. This should potentially be taken into account when determining the therapeutic strategy for AD.

Author Contributions

M.Ż., Z.B.: investigation, writing – original draft, formal analysis. P.D.: investigation, writing – original draft, visualisation. A.H.-G., R.G., A.J.-K.: investigation, writing – review. M.D.: investigation, writing – review, formal analysis, visualisation. J.G.: investigation, writing – original draft. A.Ż.: writing – review, formal analysis. T.J.: conceptualisation, funding acquisition, project administration, supervision, writing – review and editing.

Consent

All authors have read and approved the final version of this manuscript and have provided their consent for its publication in this journal.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

References

1. T. Bieber, "Atopic Dermatitis: An Expanding Therapeutic Pipeline for a Complex Disease," *Nature Reviews. Drug Discovery* 21, no. 1 (2022): 21–40.

2. N. Gori, A. Chiricozzi, F. Marsili, et al., "National Information Campaign Revealed Disease Characteristic and Burden in Adult Patients Suffering From Atopic Dermatitis," *Journal of Clinical Medicine* 11, no. 17 (2022): 5204.

3. T. Bieber, A. S. Paller, and K. Kabashima, "Atopic Dermatitis: Pathomechanisms and Lessons Learned From Novel Systemic Therapeutic Options," *Journal of the European Academy of Dermatology and Venereology* 36, no. 9 (2022): 1432–1449.

4. M. Szczepańska, L. Blicharz, and J. Nowaczyk, "The Role of the Cutaneous Mycobiome in Atopic Dermatitis," *Journal of Fungi* 8, no. 11 (2022): 1153.

5. S. H. Han, H. I. Cheon, M. S. Hur, et al., "Analysis of the Skin Mycobiome in Adult Patients With Atopic Dermatitis," *Experimental Dermatology* 27, no. 4 (2018): 366–373.

6. M. Glatz, P. P. Bosshard, W. Hoetzenecker, and P. Schmid-Grendelmeier, "The Role of *Malassezia* Spp. in Atopic Dermatitis," *Journal of Clinical Medicine* 4, no. 6 (2015): 1217–1228.

7. H. M. De Ramirez Knott, T. S. McCormick, S. O. Do, et al., "Cutaneous Hypersensitivity to *Candida albicans* in Idiopathic Vulvodynia," *Contact Dermatitis* 53, no. 4 (2005): 214–218, https://doi.org/10.1111/j.0105-1873.2005.00685.x.

8. V. Moosbrugger-Martinz, H. Hackl, R. Gruber, et al., "Initial Evidence of Distinguishable Bacterial and Fungal Dysbiosis in the Skin of Patients With Atopic Dermatitis or Netherton Syndrome," *Journal of Investigative Dermatology* 141, no. 1 (2021): 114–123.

9. H. H. Kong, J. Oh, C. Deming, et al., "Temporal Shifts in the Skin Microbiome Associated With Disease Flares and Treatment in Children With Atopic Dermatitis," *Genome Research* 22, no. 5 (2012): 850–859.

10. P. Meylan, C. Lang, S. Mermoud, et al., "Skin Colonization by *Staphylococcus aureus* Precedes the Clinical Diagnosis of Atopic Dermatitis in Infancy," *Journal of Investigative Dermatology* 137, no. 12 (2017): 2497–2504.

11. B. Schmid, A. Künstner, and A. Fähnrich, "Dysbiosis of Skin Microbiota With Increased Fungal Diversity Is Associated With Severity of Disease in Atopic Dermatitis," *Journal of the European Academy of Dermatology and Venereology* 36, no. 10 (2022): 1811–1819.

12. S. M. Edslev, P. S. Andersen, T. Agner, et al., "Identification of Cutaneous Fungi and Mites in Adult Atopic Dermatitis: Analysis by Targeted 18S rRNA Amplicon Sequencing," *BMC Microbiology* 21, no. 1 (2021): 72.

13. T. Nakatsuji and R. L. Gallo, "The Role of the Skin Microbiome in Atopic Dermatitis," *Current Allergy and Asthma Reports* 122, no. 3 (2019): 263–269.

14. J. M. Hanifin and G. Rajka, "Diagnostic Feature of Atopic Dermatitis," *Acta Dermatovener* 92 (1980): 44–47.

15. A. Y. Finlay and G. K. Khan, "Dermatology Life Quality Index (DLQI)—A Simple Practical Measure for Routine Clinical Use," *Clinical and Experimental Dermatology* 19, no. 3 (1994): 210–216.

16. C. F. Wahlgren, A. Ekblom, and O. Hagermark, "Some Aspects of the Experimental Induction and Measurement of Itch," *Acta Dermatovener* 69, no. 3 (1989): 185–189.

17. European Task Force on Atopic Dermatitis, "Severity Scoring of Atopic Dermatitis: The SCORAD Index. Consensus Report of the European Task Force on Atopic Dermatitis," *Dermatology* 186, no. 1 (1993): 23–31.

18. J. P. Leeming and F. H. Notman, "Improved Methods for Isolation and Enumeration of *Malassezia furfur* From Human Skin," *Journal of Clinical Microbiology* 25, no. 10 (1987): 2017–2019.

19. F. Sherman, "Getting Started With Yeast," *Methods in Enzymology* 350 (2002): 3–41.

20. S. Brun, J. P. Bouchara, A. Bocquel, A. M. Basile, N. Contet-Audonneau, and D. Chabasse, "Evaluation of Five Commercial Sabouraud Gentamicin-Chloramphenicol Agar Media," *European Journal of Clinical Microbiology* 20, no. 10 (2001): 718–723.

21. E. Guého, T. Boekhout, and D. Begerow, "Biodiversity, Phylogeny and Ultrastructure," in *Malassezia and the Skin: Science and Clinical Practice* (Springer-Verlag, 2010), 17–63.

22. M. Gardes and T. D. Bruns, "ITS Primers With Enhanced Specificity for Basidiomycetes Application to the Identification of Mycorrhizae and Rusts," *Molecular Ecology* 2, no. 2 (1993): 113–118.

23. T. J. White, T. D. Bruns, S. B. Lee, and J. W. Taylor, *PCR Protocols and Applications: A Laboratory Manual* (Academic Press, 1990), 315–322.

24. A. K. Gupta, Y. Kohli, R. C. Summerbell, and J. Faergemann, "Quantitative Culture of *Malassezia* Species From Different Body Sites of Individuals With or Without Dermatoses," *Medical Mycology* 39, no. 3 (2001): 243–251.

25. T. Jagielski, E. Rup, A. Ziółkowska, K. Roeske, A. B. Macura, and J. Bielecki, "Distribution of *Malassezia* Species on the Skin of Patients With Atopic Dermatitis, Psoriasis, and Healthy Volunteers Assessed by Conventional and Molecular Identification Methods," *BMC Dermatology* 14, no. 3 (2014): 1–15.

26. M. H. Sandström Falk, M. Tengvall Linder, and C. Johansson, "The Prevalence of *Malassezia* Yeasts in Patients With Atopic Dermatitis, Seborrhoeic Dermatitis and Healthy Controls," *Acta Dermatovener* 85, no. 1 (2005): 17–22.

27. V. G. Arzumanyan, O. O. Magarshak, and B. F. Semenov, "Yeast Fungi in Patients With Allergic Diseases: Species Variety and Sensitivity to Antifungal Drugs," *Bulletin of Experimental Biology and Medicine* 129, no. 6 (2000): 601–604.

28. G. Javad, M. Taheri Sarvtin, M. T. Hedayati, Z. Hajheydari, J. Yazdani, and T. Shokohi, "Evaluation of Candida Colonization and Specific Humoral Responses Against *Candida albicans* in Patients With Atopic Dermatitis," *BioMed Research International* 2015 (2015): 849206.

29. J. Savolainen, K. Lammintausta, K. Kalimo, and M. Viander, "Candida Albicans and Atopic Dermatitis," *Clinical and Experimental Allergy* 23, no. 4 (1993): 332–339.

30. Y. Choi, K. Y. Park, H. S. Han, M. K. Lee, and S. J. Seo, "Comparative Analysis of Cutaneous Fungi in Atopic Dermatitis Patients and Healthy Individuals," *Annals of Dermatology* 34, no. 2 (2022): 118–124.

31. R. D. Bjerre, J. B. Holm, A. Palleja, J. Sølberg, L. Skov, and J. D. Johansen, "Skin Dysbiosis in the Microbiome in Atopic Dermatitis Is Site-Specific and Involves Bacteria, Fungus and Virus," *BMC Microbiology* 21, no. 1 (2021): 256.

32. T. Sugita, M. Saito, T. Ito, et al., "The Basidiomycetous Yeasts Cryptococcus Diffluens and C. Liquefaciens Colonize the Skin of Patients With Atopic Dermatitis," *Microbiology and Immunology* 47, no. 12 (2003): 945–950, https://doi.org/10.1111/j.1348-0421.2003.tb03468.x.

33. E. Zhang, M. Tajima, R. Tsuboi, and T. Sugita, "Sequence Diversity of the Intergenic Spacer Region of the rRNA Gene of *Cryptococcus albidus* Isolated From the Skin of Patients With Atopic Dermatitis and Healthy Individuals," *Microbiology and Immunology* 57, no. 1 (2013): 74–76.

34. D. M. L. Saunte, G. Gaitanis, and R. J. Hay, "Malassezia-Associated Skin Diseases, the Use of Diagnostics and Treatment," *Frontiers in Cellular and Infection Microbiology* 10 (2020): 112.

35. A. Guglielmo, A. Sechi, A. Patrizi, C. Gurioli, and I. Neri, "Head and Neck Dermatitis, a Subtype of Atopic Dermatitis Induced by *Malassezia* Spp: Clinical Aspects and Treatment Outcomes in Adolescent and Adult Patients," *Pediatric Dermatology* 38, no. 1 (2021): 109–114.

36. M. Hammond, A. Gamal, P. K. Mukherjee, et al., "Cutaneous Dysbiosis May Amplify Barrier Dysfunction in Patients With Atopic Dermatitis," *Frontiers in Microbiology* 13 (2022): 944365.

37. P. S. Hamm, R. C. Mueller, C. R. Kuske, and A. Porras-Alfaro, "Keratinophilic Fungi: Specialized Fungal Communities in a Desert Ecosystem Identified Using Cultured-Based and Illumina Sequencing Approaches," *Microbiological Research* 239 (2020): 126530.

38. S. M. Rudramurthy, P. Honnavar, A. Chakrabarti, S. Dogra, P. Singh, and S. Handa, "Association of Malassezia Species With Psoriatic Lesions," *Mycoses* 57 (2014): 483–488.

39. M. Glatz, M. buchner, W. von Bartenwerffer, P. S. Schmid-Grendelmeier, M. Worm, and J. Hedderich, "*Malassezia* Spp. Specific Immunoglobulin E Level Is a Marker for Severity of Atopic Dermatitis in Adults," *Acta Dermato-Venereologica* 95 (2015): 191–196.

40. L. Storz, B. Schmid, P. P. Bosshard, P. Schmid-Grendelmeier, M. C. Brüggen, and C. Lang, "Decreased Skin Colonization With *Malassezia* Spp. and Increased Skin Colonization With *Candida* Spp. in Patients With Severe Atopic Dermatitis," *Frontiers in Medicine* 11 (2024): 1353784.

41. A. Sharma, B. Laxman, and E. T. Naureckas, "Associations Between Fungal and Bacterial Microbiota of Airways and Asthma Endotypes," *Journal of Allergy and Clinical Immunology* 144, no. 5 (2019): 1214–1227.

42. A. Baroni, I. Paoletti, E. Ruocco, M. Agozzino, M. A. Tufano, and G. Donnarumma, "Possible Role of *Malassezia furfur* in Psoriasis: Modulation of TGF-β1, Integrin, and HSP70 Expression in Human Keratinocytes and in the Skin of Psoriasis-Affected Patients," *Journal of Cutaneous Pathology* 31 (2004): 35–42.

43. J. Y. Choi, H. Kim, K. H. Min, et al., "Bacteria, Fungi, and Scalp Psoriasis: Understanding the Role of the Microbiome in Disease Severity," *Journal of Clinical Medicine* 13, no. 16 (2024): 4846.

44. Y. Koik, S. Kuwatsuka, D. Motooka, and H. Murota, "Dysbiosis of the Human Skin Mycobiome in Patients Receiving Systemic IL-23 Inhibitors," *Allergology International* 74, no. 1 (2025): 72–77.

45. D. Shuttleworth, D. Galloway, G. Boorman, and A. A. Donald, "A Double-Blind, Placebo-Controlled Study of the Clinical Efficacy of Ciclopirox Olamine (1.5%) Shampoo for the Control of Scalp Psoriasis," *Journal of Dermatological Treatment* 9 (1998): 163–167.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.