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The Assessment of Bacterial and Fungal Biodiversity in Korean Dermatomycoses Patients: A Pilot Study

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Background: Dermatophytes are the most common cause of fungal infections worldwide, having an estimated prevalence of 20% by the World Health Organization. Recent advances of sequencing and annotation of fungi explain the biodiversity evaluation and genomic identification of dermatophytes.

Objective: This study's objective is to assess the mycobiome and microbiome in different dermatomycoses and identify their pathogenic organisms using fungal genomic analyses. Besides, we wanted to see the topographical biodiversity in dermatomycoses.

Methods: Patients with dermatophyte infections confirmed by positive potassium peroxide examination were enrolled. Bacterial and fungal DNA were extracted from the skin sample. The internal transcribed spacer regions of rRNA gene and multiple variable regions in 16S rRNA gene were assessed to determine the fungal and bacterial community compositions.

Results: We profiled both bacterial and fungal communities in the fungal-infected skin. Dermatophyte infection resulted in modified bacterial microbiome. The alpha diversity showed that the bacterial diversity was relatively low in tinea cruris patients compared to other dermatomycoses patients. Nonetheless, fungal diversity was not impacted by the body site but by the causative fungal organisms. Tinea pedis, tinea corporis, and tinea cruris due to *Trichophyton* species were similar as seen in principle component analysis. Alpha diversity demonstrates the tinea corporis on the leg had the most diverse fungal mycobiome and the scalp is the least diverse area.

Conclusion: Sequencing and annotation of dermatophyte genomes of superficial skin infections will give sources that will help explain the virulence mechanisms of the dermatophyte. Understanding the pathogenesis of dermatophyte infections can help in generating effective therapeutics in the future.

Key Words: Dermatology, Fungal genomics, Microbiome, Mycobiome, Next-generation sequencing

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INTRODUCTION

The majority of skin microbiome studies have taken in a bacteriocentric view. Its only focus is on the diversity of bacterial species. However, fungi and viruses and also mites contribute to the human skin microbiome. Cutaneous fungal infections are mainly associated with two groups of fungi: one is the dermatophytes in the *Ascomycete* phylum such as the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*, and another is *Malassezia* in the *Blasidiomycete* phylum[1].

Dermatophytes are the most common cause of fungal infections in the world, resulting in treatment costs of close to half a billion dollars yearly in the USA alone. The World Health Organization estimates the global prevalence of dermatomycoses to be approaching 20%[2]. Nevertheless, dermatomycoses are considered a minor disease with only superficial infections. It has been lagged behind research on fungi associated with human mortality. And the pathogenesis of dermatophyte infections is not totally explored. So, we have no sophisticated understanding of skin fungal infection.

The diseases due to dermatophytes are generally named after the part of the infected body site rather than the causative organism. Tinea is a fungal infection of the skin, hair, or nails. The phrase is made by adding another term referring to a specific body location. Therefore, the term "tinea pedis" refers to a fungal infection of the feet. Other tinea infections include tinea capitis of the scalp or head, tinea corporis of the body or trunk, tinea cruris of the inguinal area, and tinea unguium of the nails. The terminology specifies that the focus is still not on the causative organism of dermatomycoses[3].

Recent developments of next-generation sequencing technologies and the microbiome of several body sites, such as the skin, have been characterized and have explained the mechanisms of the virulence factor, biodiversity assessment, and genomic identification of the dermatophyte. Molecular markers for fungi, such as the rRNA gene, including 18S, 28S, internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2) regions, and the 16S rRNA gene for

Table 1. Patients with dermatophyte infections

Patient code	Gender	Age	Lesion of sample collections	KOH	Fungus culture	Diagnosis	Comorbid skin disease
Foot 1 (psoriasis)	M	45	Foot	+	Growth of mold Fungi → *Trichophyton rubrum*	Tinea pedis Onychomycosis	Psoriasis
Foot 2	F	66	Foot	+	Growth of mold Fungi → *Trichophyton rubrum*	Tinea pedis	None
Foot 3	M	48	Foot	+	No growth in 4 weeks culture	Tinea pedis Onychomycosis	None
Leg	M	79	Lower leg	+	Growth of *Keissleriella* sp., possibly contaminations	Tinea corporis Onychomycosis	None
Groin 1 (atopic der)	M	16	Groin	+	Growth of mold Fungi → *Trichophyton rubrum*	Tinea cruris	Atopic dermatitis
Groin 2	M	69	Groin	+	No growth in 4 weeks culture	Tinea pedis, Tinea cruris Onychomycosis	None
Groin 3	M	42	Groin	+	Growth of mold Fungi → *Trichophyton rubrum*	Tinea cruris, Onychomycosis	None
Buttock 1	M	69	Buttock	+	Growth of mold Fungi → *Cladosporium* sp.	Tinea corporis	None
Buttock 2	M	57	Buttock	+	Growth of mold Fungi → *Trichophyton rubrum*	Tinea corporis	None
Scalp	M	7	Scalp	+	Growth of mold Fungi → *Trichophyton* sp.	Tinea capitis	None

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bacteria, are in sequence and classify these communities.

In this manuscript, we assess the mycobiome and microbiome at the same time in different dermatomycoses and determine the pathogenic organisms with the use of fungal genomic analyses. Ultimately, we wanted to know the topographical biodiversity in dermatomycoses.

MATERIALS AND METHODS

1. Subjects

Dermatophyte-associated dermatologic diseases, including tinea capitis, tinea cruris, tinea corporis, and tinea pedis, were assessed using genomic sequencing methods. Ten patients with dermatophyte infections confirmed by positive potassium peroxide (KOH) examination were enrolled in this study. The microbiome and mycobiome of the dermatophyte-infected skin were assessed. The samples were placed into a lysis buffer using a sterile cotton swab and a sterile disposable surgical blade. Bacterial and fungal DNA were extracted from the skin sample using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) and MasterPure Yeast DNA purification kit (Epicentre), respectively.

2. Primers

To identify the fungal and bacterial community compositions, the internal transcribed spacer (ITS) 2 regions were amplified from purified genomic fungal DNA with the use of primers 5.8S-F (5'-GATGAAGAACGYAGYRAA-3') and 28S-R (5'-TCCTCCGCTTATTGATATGC-3'). And multiple variable regions in 16S rRNA gene from purified genomic bacterial DNA were assessed with the use of an ion torrent sequencing (Thermo Fisher Scientific).

RESULTS

1. Demographics of patients

Patients who came to Uijeongbu St. Mary's Hospital Dermatology Clinic due to dermatomycoses from last August to September 2019 were enrolled in this study. They were diagnosed with tinea corporis, tinea capitis, tinea pedis, and tinea cruris, confirmed with potassium hydroxide tests.

Among the 10 patients with dermatomycoses, 9 were males and 1 patient female. The mean age of the patients was 49.8 ± 22.3, including one child with tinea capitis and one adolescent with tinea cruris. The 16-year-old boy had atopic dermatitis, and one 45-year-old patient had psoriasis (Table 1).

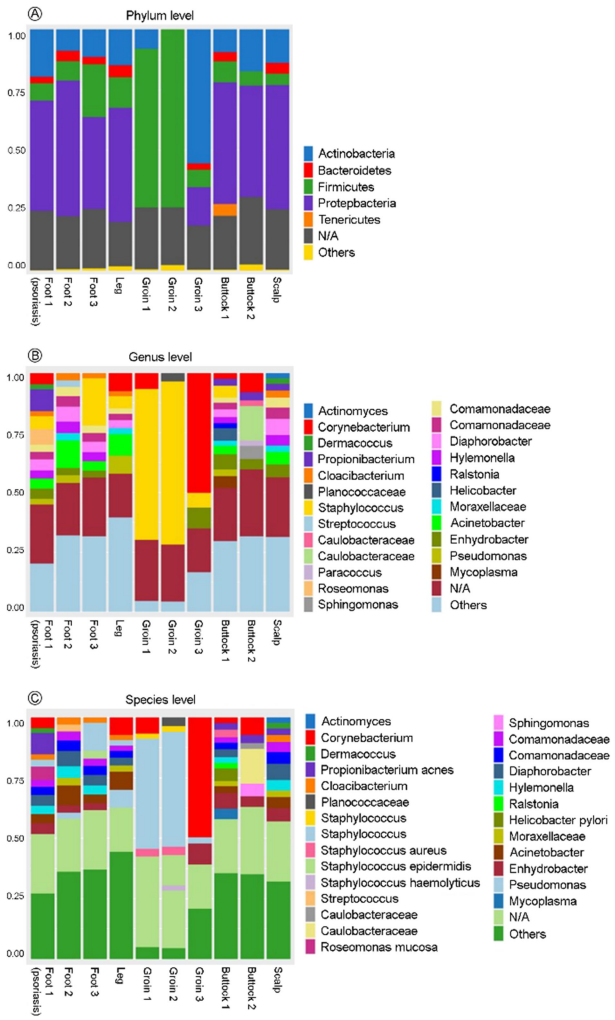

Fig. 1. Metagenome analysis of bacteria

2. Bacterial taxonomical analyses

At the phylum level, *Proteobacteria* had the largest proportion of microbiome in tinea pedis, tinea corporis, and tinea capitis patients. However, in tinea cruris patients, *Firmicutes* and *Actinobacteria* are predominant in the groin area (Fig. 1A). At the genus level, *Staphylococcus* and *Corynebacterium* are relatively predominant in tinea cruris patients. Other body sites seem to have bacterial diversity (Fig. 1B). At the species level, *Staphylococcus* increased in tinea cruris patients. *Propionibacterium acne* is indicated in tinea pedis patients

with psoriasis, while it is not shown in other tinea pedis patients or tinea corporis in the lower legs (Fig. 1C).

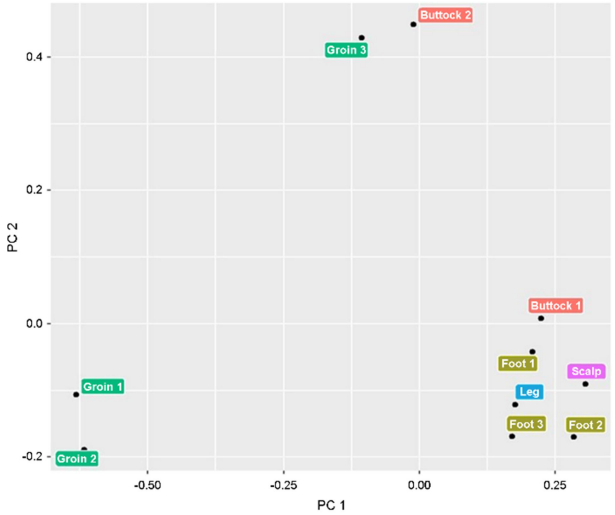

Fig. 2. Principle component analysis of bacteria. Bacterial microbiome of tinea cruris is different from those of other dermatomycoses. This principle component analysis based on Bray-Curtis dissimilarities.

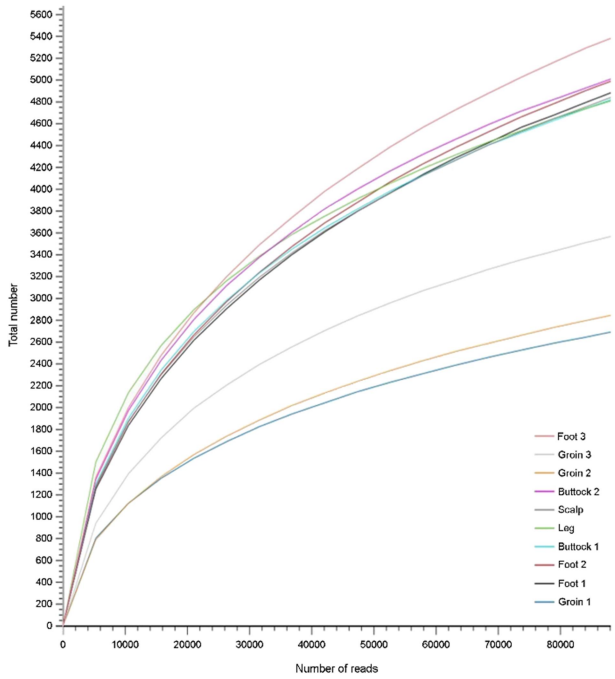

Fig. 3. Alpha diversity of bacteria. The bacterial diversity was relatively low in tinea cruris patients compared to other site dermatomycoses.

3. Principle component analysis and alpha diversity of bacterial microbiome

The principle component analysis exhibited that the bacterial microbiome of tinea cruris is varied from those of other dermatomycoses (Fig. 2). Alpha diversity also showed that the bacterial diversity was relatively low in tinea cruris patients in comparison with other site dermatomycoses (Fig. 3).

4. Taxonomical analyses

The fungal diversity of dermatomycoses patients was taxonomically classified at the phylum level. *Ascomycota* predominated in nine patients, and *Basidiomycota* is chiefly presented in one patient who had been diagnosed with tinea pedis on the foot and had comorbid psoriasis lesions on the

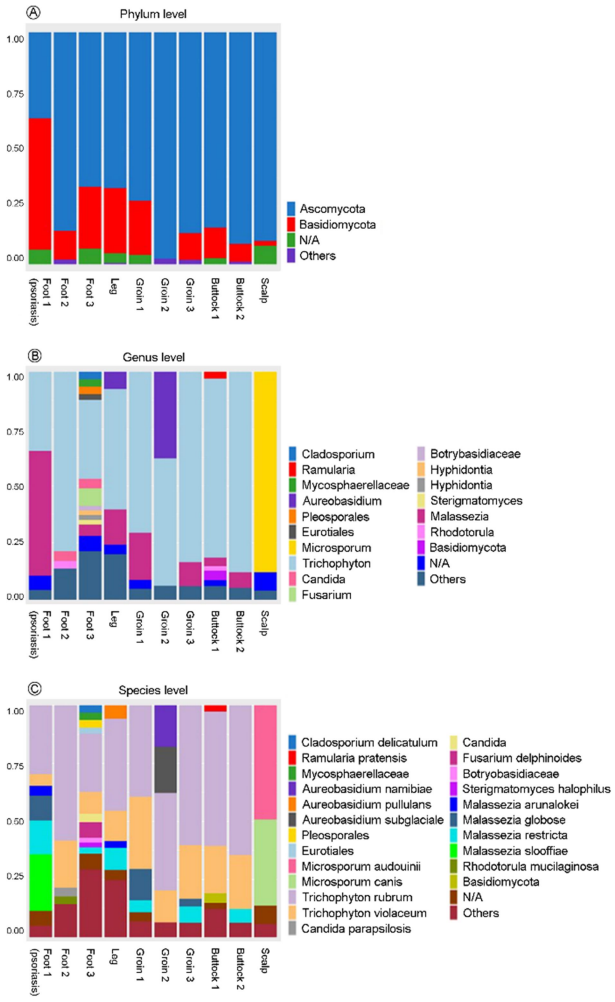

Fig. 4. Metagenome analysis of fungi

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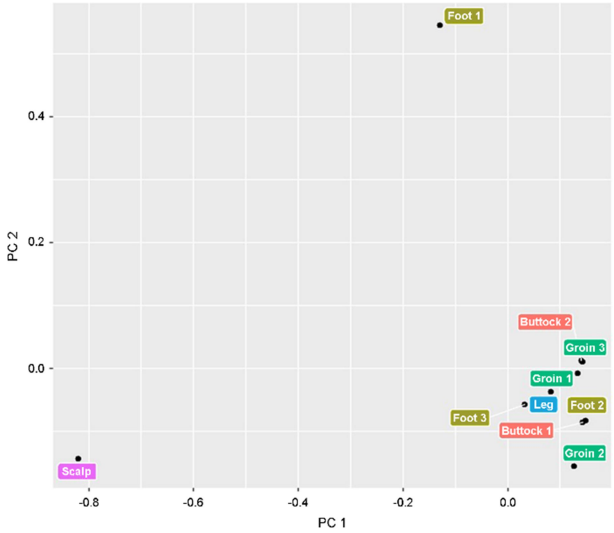

Fig. 5. Principle component analysis of fungi. Fungal mycobiome shows the similarity between the dermatomycoses patients except tinea capitis and tinea pedis with psoriasis.

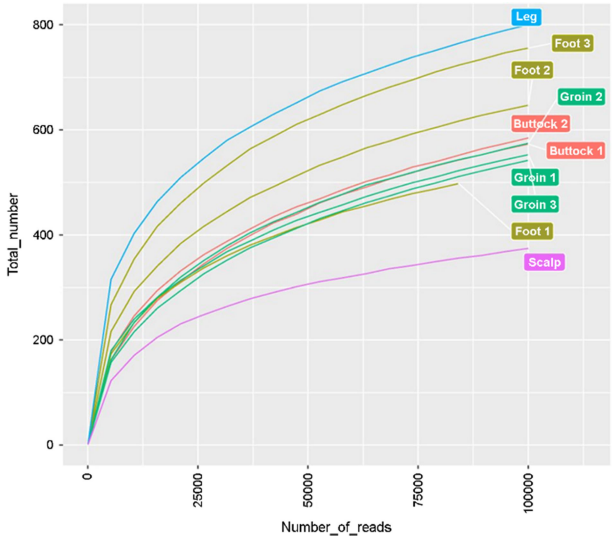

Fig. 6. Alpha diversity of fungi. Alpha diversity shows the tinea corporis on the leg had most diverse fungal mycobiome and scalp is the least diverse area.

leg (Fig. 4A). At the genus level, *Microsporum* prevailed in a tinea capitis patient, and *Trichophyton* was the most predominant genus in other tinea cruris, tinea pedis, and tinea corporis patients excluding one who had been diagnosed with psoriasis and tinea pedis on the foot, which showed *Malassezia* to be predominant (Fig. 4B). At the species level, the fungal composition of the tinea pedis patient with comorbid psoriasis exhibited that the most prevalent and pathogenic fungal organism was *Trichophyton rubrum* (more than 25%); however, *Malassezia arunalokei*, *Malassezia globosa*, *Malassezia restricta*, and *Malassezia slooffiae* had large proportions in the previously mentioned patient in comparison with other patients. The pathogenic organisms in a 7-year-old tinea capitis patient were *Microsporum audouinii* and *Microsporum canis*. Other dermatomycoses patients exhibited a large proportion of *Trichophyton rubrum* (Fig. 4C).

5. Principle component analysis and alpha diversity of fungal mycobiome

The principle component analysis indicated that *Trichophyton* rubrum-infected dermatomycoses differed from the *Microsporum*-infected tinea capitis patient (Fig. 5). Alpha diversity shows that the tinea corporis on the leg had the most diverse fungal mycobiome and the scalp is the least diverse area (Fig. 6).

DISCUSSION

This study described that the fungal diversity was similar in *Trichophyton rubrum*-infected dermatomycoses regardless of body sites. The modified biodiversity of bacterial microbiome might be explained by the damaged epidermal barrier due to dermatophyte infection. The condition of patients' skin and comorbid cutaneous diseases can impact the microbiome and mycobiome of the skin.

According to previously reported microbiome of the healthy skin, most skin bacteria comprise four phyla: *Actinobacteria* (52%), *Firmicutes* (24%), *Proteobacteria* (17%), and *Bacteroidetes* (7%). At the genus level, *Corynebacterium* in phylum *Actinobacteria*, *Propionibacterium* in phylum *Actinobacteria*, and *Staphylococcus* in phylum *Firmicutes* are found on the healthy skin[4]. When the body skin sites are split into three categories, sebaceous site, moist site, and dry site, the topographical diversity of microbiome was observed. Sebaceous sites including the scalp show the lowest bacterial diversity and isolate mainly *Propionibacterium* species and *Staphylococcus* species. And moist areas including the groin and foot preferentially have *Corynebacterium* species. Dry areas such as the buttock and leg have the most diverse microbial community with a greater prevalence of β-*Proteobacteria* and *Flavobacteriales* species[4,5].

However, in this study, the modified microbiome was observed in dermatomycoses patients. The proportion of

Staphylococcus species in microbiome increased on the inguinal area of tinea cruris patients. In normal healthy skin, the groin area is considered to have a moist condition, and *Corynebacterium* species are predominant in this site. That discrepancy might be influenced by the impaired epidermal barrier function of tinea cruris infection. *Propionibacterium acne* is predominant in greasy areas, for instance, the scalp, while the proportion of *Propionibacterium acne* was relatively low on the scalp of tinea capitis patients. Unexpectedly, *Propionibacterium acne* was seen in tinea pedis patients with psoriasis, whereas it was not shown in other tinea pedis patients. That might be explained by the topical ointment use in psoriasis patients.

In comparison to previously reported microbiome of the healthy skin, the microbiome of the dermatomycoses is more diverse and different than of the healthy skin. That might be the pathogenic role of fungal infection that damages the skin barrier function. Dermatophyte infection resulted in modified bacterial microbiome. The alpha diversity showed that the bacterial diversity was relatively low in tinea cruris patients in comparison with other site dermatomycoses.

Meanwhile, both *ascomycetous* and *basidiomycetous* fungi were determined in normal healthy skin. The genus *Malassezia* is predominant in all core body and arm sites[6]. However, in this study, *Malassezia* only predominated at the foot of the tinea pedis patient who had been treated with comorbid psoriasis. The tinea capitis patient principally exhibited colonization of *Microsporum* spp. Other dermatomycoses patients exhibited predominant colonization of *Trichophyton rubrum*.

In this study, fungal diversity was impacted not by body sites but by the causative fungal organisms. Tinea pedis, tinea corporis, and tinea cruris due to *Trichophyton* species exhibited a similarity, using the principle component analysis. Alpha diversity shows that the tinea corporis on the leg had the most diverse fungal mycobiome and the scalp is the least diverse area.

This study has various limitations as follows: The analysis was only of patients, with no healthy control comparison. We also did not compare the microbiomes before and after antifungal treatment. The number of patients was too small to find significant conclusions.

On the basis of these results, we propose that the causative organisms of dermatomycoses are more important than the infected body sites. There are still several things to be analyzed for cutaneous fungal diseases using genomic studies. Sequencing and annotation of dermatophyte genomes of superficial skin infections will give sources that will help in explaining the mechanisms of virulence of the dermatophyte. The understanding of pathogenesis of dermatophyte infections can design the effective therapeutics in the future.

CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

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