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REVIEW

Topographical and physiological differences of the skin mycobiome in health and disease

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ABSTRACT

Skin constantly encounters external elements, including microbes. Culture-based studies have identified fungi present on human skin and have linked some species with certain skin diseases. Moreover, modern medical treatments, especially immunosuppressants, have increased the population at risk for cutaneous and invasive fungal infections, emphasizing the need to understand skin fungal communities in health and disease. A major hurdle for studying fungal flora at a community level has been the heterogeneous culture conditions required by skin fungi. Recent advances in DNA sequencing technologies have dramatically expanded our knowledge of the skin microbiome through culture-free methods. This review discusses historical and recent research on skin fungal communities – the mycobiome – in health and disease, and challenges associated with sequencing-based mycobiome research.

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Introduction

Skin is the outermost compartment of the body and often the first point of contact for an extensive number of environmental microbes. The skin surface is a unique niche, serving as a habitat for a distinct community of microorganisms.[1] While metagenomic analyses have shown that the majority of annotated reads from samples obtained from most skin sites are bacterial, some sites may have relatively high proportions of fungal or viral sequences.[2,3] Understanding skin fungal communities as well as investigating interactions between bacterial and fungal communities are essential, especially given the association of fungi with various skin disorders, such as seborrheic dermatitis, atopic dermatitis, and dermatophytosis.[4-9]

Culturing has long been used to isolate and identify fungi on skin. However, this approach may not fully capture the diversity and/or relative composition of fungal communities, as optimal culture conditions and growth rates vary widely between species.[10] Several molecular approaches such as restriction fragment length polymorphism (RFLP) analyses have been used to complement culturing; however, these low-throughput methods only take into account a portion of the community and likely underestimate the diversity of fungi present. Development of high-throughput sequencing techniques and microbiome analysis methods are revolutionizing our understanding of the "mycobiome"—here defined as the overall fungal habitat, including fungi, "their genomes (i.e. genes), and the surrounding environmental conditions."[11] This review focuses on efforts to examine the skin mycobiome in healthy individuals and alterations associated with various skin conditions and disorders.

"Normal" skin mycobiome

Cultivation methods recover primarily *Malassezia* (formerly known as *Pityrosporum*),[12] as well as occasionally *Candida* and a variety of other species on healthy human skin.[13] Initial studies used culture-based techniques to speciate *Malassezia*, relying on morphology and lipid dependence to differentiate isolates[12] and identifying new species based on distinct physiological and biochemical features.[14] Culturing surveys commonly have isolated *M. globosa, M. restricta*, and *M. sympodialis* from healthy human skin.[14] However, cultivation-based studies can be biased by culture media and growth conditions, so results from different studies may not be comparable. *Malassezia* isolates can require different conditions for optimal growth, and some species (such

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as *M. furfur* and *M. sympodialis*) grow more quickly than others (*M. restricta* and *M. obtusa*), which may skew the relative abundances of species observed.[15]

Molecular methods have facilitated investigations of *Malassezia spp*. Genomic differences, which have been shown to correspond to morphological distinctions, allow for inter-species resolution.[16] Analyses based on nested PCR or RFLP have suggested that *M. globosa, M. restricta, M. sympodialis*, and *M. furfur* inhabit the skin of healthy individuals.[17,18] More recent studies performing culture-independent sequencing analyses of skin fungal communities rely on amplicon sequencing using fungi-specific phylogenetic markers such as the 18S rRNA gene, internal transcribed spacers, and the 28S rRNA gene.[19] Researchers have used a single region or combination of regions, with primers targeting the D1/D2 region of the 28s rRNA gene,[20] the 18S rRNA gene with the 5.8S/ITS2 region for *Malassezia* speciation,[21] ITS1-5.8S-ITS2 and the 28S rRNA gene,[22] or ITS1 and the 18S rRNA gene.[23] These cultivation-independent studies from individuals residing in varying geographic regions also showed *Malassezia* predominates on most skin sites[20-22,24] except the feet.[23] Eleven of the fourteen *Malassezia* species have been identified by sequencing swabs from healthy human skin, indicating the range of colonization possible.[23] Here, we discuss the distribution of skin fungi in association with body site, age, and gender.

Body site

Quantitative culturing has shown that *Malassezia* colonization is particularly high in sebaceous sites such as the scalp and the forehead.[25] Several *Malassezia* species are present in statistically significantly different proportions at spatially distinct body sites. *M. globosa* was present in higher abundances on head sites (scalp and forehead) and in lower abundances on the back, while relative abundances of *M. sympodialis* were distributed in the inverse pattern.[26] Results from culture-independent studies also indicated that fungal communities are strongly influenced by body location, with different sites harboring distinct populations.[20,23] Using ITS1 sequencing, *M. restricta* and *M. globosa* patterns were similar among different individuals sampled at the same site. *M. restricta* predominated on head and facial sites whereas *M. globosa* was predominant on trunk sites. Other body sites demonstrated more mixed *Malassezia* communities, and feet were populated by diverse fungal communities including *Aspergillus, Cryptococcus, Rhodotorula*, and *Epicoccum*[23] (Fig. 1).

The site selectivity of *Malassezia* species may be explained by genomic differences within the *Malassezia* genus that can affect niche adaptation. The *Malassezia* genus lacks the fatty acid synthase gene, so species are lipid-dependent, explaining their predominance on sebaceous sites. However, different species vary in their lipase and phospholipase genes with resultant variation in lipid preferences.[27] Given that lipid profiles vary at different skin sites,[28,29] these preferences may underlie the observed adaptation to different sites, although more research is needed to clarify how genetics may drive niche preference.

Age

Shifts in fungal colonization occur with age, with young subjects colonized by lower levels of *Malassezia* and colonization generally increasing in adolescence to adulthood.[15,26,30] Analysis based on ITS1 sequencing has also shown low relative abundance of *Malassezia* accompanied by high fungal diversity on the skin of preadolescent children aged 8–13 y as compared to adults[31] (Fig. 1). These observed shifts may be explained by the increase in sebaceous activity during adolescence which provides a more favorable niche for lipophilic *Malassezia* to colonize.[32] The variation in fungal communities across different age groups may contribute to the age predilection of several fungal infections. For example, tinea capitis and tinea corporis are typically caused by a variety of non-*Malassezia* fungi and are more prevalent in children, whereas tinea versicolor, associated with *Malassezia* spp., is more common in adults.[6,7,33]

At the species level, culture-based methods have shown that *M. globosa* predominated in children whereas *M. sympodialis* was typically absent; in contrast, *M. globosa* was less common than *M. sympodialis* in adults.[26] Culture-independent analyses also showed that *M. globosa* was less abundant in adult subjects, particularly at facial sites, with *M. restricta* predominating.[15,23,31] Changes in the composition of the secreted lipids may explain these shifts over time, given the different lipid preferences of individual species.[27,34]

Gender

Sugita et al. noted that female subjects had a reduced abundance of *Malassezia* at the age of 19–29 years as compared to male subjects of the same age or female subjects of different ages. The authors attributed this finding to the prevalent use of cosmetics, which contain compounds that may inhibit cutaneous fungal growth.[15] In atopic dermatitis patients, culture-independent methods showed that females had a statistically significantly reduced abundance of *Malassezia* spp. on their faces, but not their upper trunk skin, as compared to males.[35] However, another study failed to identify significant differences in *Malassezia* colonization based on gender,

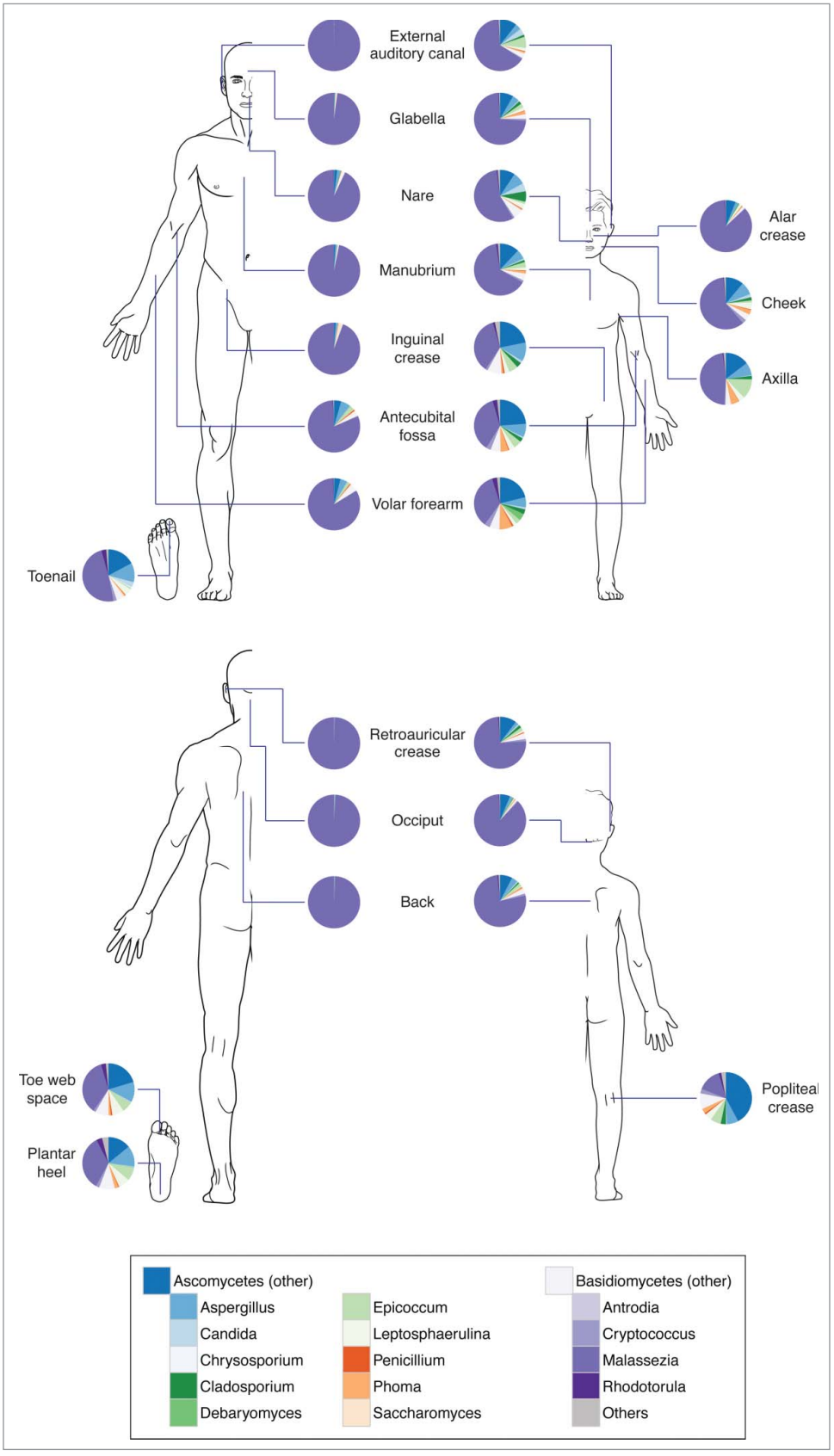

Figure 1. Composition of skin fungal communities distributed over various skin sites and in different age groups. Phylum- and genus-level classification of fungi colonizing skin of healthy adults (left, age 18–39 years) and children (right, age 8–13 years). Data adapted from Ref.[27].

although results were based on culturing and cosmetic use was not discussed.[36]

Mycobiome and pathogenesis of skin diseases

The major commensal skin fungi: Malassezia spp

A goal of studying the human skin mycobiome is to understand the role of fungal communities in health and disease. In addition to being the most prevalent fungus identified on human skin, the genus *Malassezia* has been extensively studied to understand its connection to skin diseases. Various reports have proposed that *Malassezia* spp. are associated with several dermatologic conditions, including tinea versicolor (also known as pityriasis versicolor), atopic dermatitis, and seborrheic dermatitis.[4,5]

Tinea versicolor is characterized by the appearance of round lesions that are hyper-or hypopigmented than the surrounding skin.[4] Lesions preferentially appear on sebaceous skin areas, such as the back and chest. Studies of the pathogenesis of tinea versicolor typically implicate *Malassezia*. Reports have shown that affected lesions have higher loads of *M. globosa, M. sympodialis*, and *M. furfur* as compared to non-lesional sites and the skin of healthy individuals.[37-40] Mechanistically, it has been suggested that *Malassezia* proteins such as malassezin and pityriacitrin contribute to hypo- or hyperpigmentation of the lesion by inducing apoptosis of melanocytes and directly absorbing UV light.[41-43]

Atopic dermatitis is a common inflammatory skin disease characterized by chronic relapsing itchy red skin. Many factors contribute to the development and progression of atopic dermatitis, including genetics, skin barrier, environmental factors, and skin bacteria.[44-47] Although several culture-based studies have described lack of association between atopic dermatitis and *Malassezia* spp.,[25,40,48,49] others have shown an association between *Malassezia* and atopic dermatitis. A molecular-based study (nested PCR) showed that *Malassezia* spp. are more frequently detected and are more diverse in patients than healthy subjects.[17] Some studies also demonstrated higher *Malassezia*-specific serum IgE in atopic dermatitis patients[50-52] and systemic antifungal ketoconazole treatment in patients with relatively high *Malassezia*-specific IgE levels was associated with clinical improvement.[50] Other studies have described IgE or reactive T cells that recognize *Malassezia* antigens, e.g. HSP70, MnSOD, and thioredoxin, in patients with atopic dermatitis.[4,53-55]

Seborrheic dermatitis is characterized by recurrent, relapsing inflammation with yellowish greasy scaling observed in sebaceous areas of the head and trunk, specifically the scalp (dandruff). Several studies have linked seborrheic dermatitis and dandruff with *Malassezia*. Culturing showed *Malassezia* spp. were more frequently isolated from the lesions of seborrheic dermatitis.[40] Also, data suggest a causal link between *Malassezia* and dandruff, since various antifungal reagents—zinc salts, selenium salts and azoles—are highly effective in the treatment of dandruff.[56,57] Improvement of the disease by anti-dandruff shampoo use is accompanied by reduced *Malassezia* levels.[57] Although the role of *Malassezia* in this disease is not well understood, the higher prevalence of seborrheic dermatitis in immunodeficient HIV patients[58-60] (10–30 times higher than the general population) suggests an immune response against *Malassezia* might be important for controlling the disease.

While culture-based studies have shown that *Malassezia* spp. are more frequently isolated from the lesions of tinea versicolor, atopic dermatitis, and seborrheic dermatitis, recent culture-independent analyses have shown that *Malassezia* are a part of the normal skin flora.[23,27,61] Therefore, *Malassezia* spp. are likely to be opportunistic microorganisms that may elicit disease with changes in host immunity or growth phase—yeast versus mycelial form—of the fungus.[25,62] Higher levels of anti-*Malassezia* antibodies have been observed in the serum of atopic dermatitis[50] and seborrheic dermatitis[63] patients (IgE and IgG, respectively). Microscopic studies illustrated that the mycelial forms of *Malassezia* were frequently observed in lesional sites of tinea versicolor, but the yeast form was present on non-lesional skin and on healthy controls.[37,64,65] In addition, several *Malassezia* metabolites or virulence factors, e.g., indole compounds[41] and lipases,[66] have been proposed to play roles in the pathogenesis of certain skin diseases. Some indole derivatives from *M. furfur* have been shown to act as aryl hydrocarbon receptor agonists involved in various cellular processes such as immune signaling, cell cycle, and apotosis.[67] More recently, whole genome sequencing of several *Malassezia* species was performed, which provides insights into niche selection and virulence.[27]

Mycobiome alteration and disease

While most studies examining the relationship between microbes and disease focus on culturing and implicate a single microbial species, microbial communities can be studied at a global level with sequencing-based approaches. In general, microbiome studies have focused primarily on bacteria with less of an emphasis on fungi. Studies of the bacterial skin communities have shown that diversity is inversely correlated with disease severity in atopic dermatitis and psoriasis.[47,68,69] In contrast, higher fungal diversity has been associated with atopic dermatitis[61] and psoriasis[70] as compared to controls. Atopic dermatitis patients also had increased relative abundances of *Candida* spp., *Cryptococcus* spp. and *Malassezia globosa* in comparison to healthy individuals,

and psoriatic skin lesions had increased relative abundances of *Malassezia restricta*. However, the relevance of these mycobiome diversity and community differences to disease pathophysiology remains unknown. Therefore, additional studies in these and other skin diseases with proper controls will be important for understanding mycobiome shifts.

Role of host factor in skin mycobiome homeostasis and disease

Host factors, such as genetics and immune status, can influence microbiome composition and, in turn, diseases. Patients with primary immunodeficiency syndromes often suffer from recurrent infections, including fungal infections.[71-73] Chronic candidiasis is frequently observed in patients with mutations in immune modulatory genes, such as *AIRE* (autoimmune regulator), dectin-1 (C-type lectin receptor), and *CARD9* (Caspase recruitment domain family 9).[74-76] Signal transducer and activator of transcription 3 (STAT3) is a key transcription factor involved in immune signaling, and is activated by interleukin-6 (IL-6), IL-10, IL-23, IL-21 and IL-11.[77] Heterozygous mutations in *STAT3* causes hyper-IgE syndrome (HIES), a primary immunodeficiency disease characterized by high serum IgE, atopic dermatitis-like symptoms, and systemic infections.[78-80] Oh et al. demonstrated that STAT3-HIES patients exhibited increased mycobiome richness, with reduced *Malassezia* and increased *Aspergillus* and *Candida* even on unaffected skin.[81] This observation corresponds to clinical manifestations of STAT3-HIES, which often include fungal infections such as mucocutaneous candidiasis and pulmonary *Aspergillus* and *Scedosporium* infections. Since monogenic disorders that alter host immune function can result in susceptibility to fungal colonization and infection, studying mycobiome alterations in this context may enhance our understanding of host-microbial interactions.

Abnormal fungal infections can also occur in patients with acquired immunodeficiency, such as individuals with AIDS or those on immunosuppressive medications. The incidence of fungal infections has surged in the last few decades with the annual number of deaths from invasive mycoses in the United States increasing about 320% from 1980 to 1997,[82,83] mainly due to the rise in immunocompromised individuals in the population.[84-87] Cutaneous fungal infections in immunocompromised patients are caused by common opportunistic pathogens such as *Candida, Aspergillus*, and *Cryptococcus*. However, severely immunocompromised patients can sometimes be infected by fungi that usually do not colonize humans, such as *Trichosporon* spp, *Fusarium* spp., and *Emmonsia* spp..[88] Although various fungal infections were reported in acquired immunodeficiency patients, no skin mycobiome studies have been done in these individuals. Individuals with acquired immunodeficiency may be colonized by a diverse group of fungi, similar to the primary immunodeficiency patients.[81] Overall, host immune responses appear to be critical for maintaining a "healthy" fungal flora and preventing opportunistic infection; understanding the mycobiome alterations that occur in immunodeficient individuals may provide insights into the source and/or pathogenesis of these fungal infections.

Further directions of sequence-based mycobiome studies

In recent decades, our understanding of the microbiome has expanded with the development of and rapid technical advances in sequencing technology. Large-scale studies, such as the National Institutes of Health Common Fund's Human Microbiome Project, have not only provided profiles of the microbial communities at various body sites in healthy volunteers, but have also standardized pipelines for analysis.[1] However, more investigations of the fungal communities on various body sites and standardized methods for studying the mycobiome are needed.

Many fungi require specific cultivation conditions for optimal growth and isolation, hampering precise quantitative analyses; sequencing-based approaches enable culture-free and high-throughput investigations of the skin fungal communities. However, there are several challenges and limitations to consider for sequencing-based mycobiome analysis. Given the low microbial biomass present on skin, samples are particularly prone to contaminants showing up in sequencing results. Sample handling and reagent contamination can affect results, so negative controls must be processed in parallel to confirm sample integrity.[89] Additionally, standardized and high-yield protocols for extracting microbial DNA from the skin mycobiome are important. Use of physical cell disruption method (bead-beating) combined with chemical methods (detergents and enzymes) during DNA extraction facilitates breaking cell walls of fungi and accessing more fungal genomes, and can possibly lessen the bias toward a particular taxonomic group.[90]

Currently, targeted amplicon sequencing is widely used for mycobiome analysis; therefore, the selection of marker genes for taxonomic classification and the robustness of reference databases is critical for developing standardized mycobiome pipelines. Commonly used marker genes are internal transcribed spacer regions (ITS1/2) and rRNA (18S, 5.8S and 28S rRNA) genes.[21,22,61,91] Advantages and disadvantages for each

marker are evident: ITS exhibits high sequence variation among different taxa, providing greater taxonomic resolution but poor alignment. The 18S rRNA gene is well conserved and easier to align for better phylogenetic analysis, but allows for less taxonomic resolution. Although current reference databases for ITS1 (UNITE,[92] ITSoneDB[93]) and 18S rRNA (SILVA[94]) are useful for classification, these databases include a limited number of fungi. It is estimated that approximately 1.5–5.1 million fungal species exist, whereas existing databases include less than 100,000 species.[95,96] Bioinformatic methods that address these difficulties are under active development. Fouquier et al. developed a hybrid pipeline that uses ITS1 and 18S rRNA sequences together to ensure both taxonomic resolution and phylogenetic analysis,[97] and a newer ITS1 database (ISHAM[98]; International Society of Human and Animal Mycology) and sequence analysis pipeline specific for mycobiome studies (CloVR-ITS[99]) are available.

An additional limitation of sequencing-based approaches includes the inability to differentiate between live and dead organisms. Several studies have addressed this challenge by developing methods that prevent DNA amplification and sequencing of dead cells.[100-101] Furthermore, longitudinal surveys in the same study population can help to distinguish between long-term colonizers vs. transient microorganisms.

Several studies of the human microbiome have suggested that a homeostatic balance exists between a host and its commensal microbes. Disturbance of this homeostasis may result in disease; therefore, investigating skin mycobiome-host interactions is important for understanding the pathophysiology of various skin conditions and infections. Utilizing various "omic" approaches will be important for understanding and deciphering host-microbe and microbe-microbe interactions. Likewise, inter-kingdom interactions on skin is another intriguing area of research. Targeted species analysis has shown that there are diverse interactions between fungi and bacteria. For example, *Candida albicans* and *Staphylococcus aureus* can co-colonize and form mixed biofilms, potentially protecting them from antifungal and antibiotic reagents.[102] Additionally, *Pseudomonas aeruginosa* was shown to inhibit *C. albicans* colonization on burn wounds.[103] Therefore, inter-kingdom interactions within the microbiome are another crucial area to be explored. Metagenomic shotgun sequencing currently enables examination of the bacterial communities, mycobiome, and virome in a single sequencing reaction. Development of additional methods and pipelines for incorporating proteomics, transcriptomics, metabolomics, and lipidomics will widen our perspective regarding the biological and clinical implications of the mycobiome. Ultimately, studying skin mycobiome-host interactions will expand our understanding of disease pathogenesis and ability to find novel therapies for various skin conditions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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