Short Report

Malassezia Display a Hyphae-like “Spaghetti-and-Meatballs” Configuration in Keratotic Plugs

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ABSTRACT

Malassezia are lipophilic yeasts in the skin microbiome that abundantly colonize all parts of human skin except for the soles of the feet. Fungal microbiome analysis of keratotic plugs from the noses of 10 healthy individuals identified Malassezia restricta as the predominant species, followed by Malassezia globosa. Malassezia hyphae were observed in 5 of the 10 individuals. The hyphae were curved and thick-walled with spherical thick-walled and grouped blastoconidia, described as a “spaghetti-and-meatballs” configuration. In this study, we observed Malassezia hyphae in keratotic plugs of healthy subjects, although abundant Malassezia hyphae have previously only been observed in lesional sites of patients with pityriasis versicolor.

Key words: hyphae, keratotic plug, Malassezia, “spaghetti-and-meatballs” configuration

A wide variety of microorganisms are present on human skin, and bacterial microbiomes generally vary at different body sites, e.g., Cutibacterium acnes and Staphylococcus epidermidis are abundant at sebaceous sites, while Corynebacterium tuberculostearicum and Staphylococcus hominis are abundant at moist sites. By contrast, Malassezia species are found at all body sites, except the foot soles. As Malassezia species are lipophilic fungi, they dominate in areas containing large sebaceous glands, such as the scalp, face, chest, and back. Malassezia species cause or exacerbate pityriasis versicolor, seborrheic dermatitis, folliculitis, or atopic dermatitis and are normal commensal microorganisms in healthy individuals. Eighteen species have been identified in the genus Malassezia, of which nine have an affinity for humans, and the remaining nine have an affinity for non-human animals. Malassezia cells are globose, ovoid, or cylindrical, and hyphal cells are rare in culture. However, hyphae with clusters of blastoconidia, known as a “spaghetti-and-meatballs” (SM) configuration, may occur, particularly on skin scales of pityriasis versicolor lesions. Abundant hyphae are not observed on the skin of healthy individuals or patients with Malassezia-related skin diseases other than pityriasis versicolor. However, we found abundant Malassezia hyphae in keratotic plugs during skin microbiome analysis in this study.

This study involved 10 healthy individuals (6 males and 4 females, average age 22.3 ± 1.1 years). The study protocol was reviewed and approved by the Institutional Review Board of our institution, and written informed consent was obtained from each individual (Approval Number 201903). Ten keratotic plug samples from each individual were taken from the noses of the subjects using Biore Deep Cleansing Pore Strips (Kao Corporation, Tokyo, Japan) according to the manufacturer’s instructions (Fig. 1). Immediately after collection, each keratotic plug on the strip was cut in half with a sterilized disposable knife, and segments for each subject were pooled. Half of the segments were used for DNA extraction and microbiome analysis, and the other half used for morphological observation. Microbiomes were analyzed following the method of Suzuki et al. Briefly, fungal rRNA genes were amplified by PCR, and pooled amplicons were prepared for sequencing using a Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) and sequenced on a MiSeq platform using MiSeq ver. 3 Reagent Kits (Illumina), as per the manufacturer’s instructions. Microbiome analysis was performed using the QIIME 2 pipeline (https://qiime2.org).

A total of 907,475 reads were analyzed to elucidate the
fungal microbiome of the keratotic plugs. *Malassezia* species (85.3 ± 6.6%) predominated, followed by *Candida albicans* (9.1 ± 2.8%) and *Rhodotorula mucilaginosa* (2.5 ± 0.0%). The three taxa accounted for 96.9 ± 3.8% of the fungal microbiome (Fig. 2). The ratio of *Malassezia restricta* and *Malassezia globosa* to the overall *Malassezia* colonization was 87.6 ± 6.9% and 9.0 ± 0.9%, respectively (Fig. 2). Keratotic plugs were stained with calcofluor-white, and fungal morphological observation was performed with a fluorescence microscope. Abundant hyphae were observed from the samples of five subjects. Fig. 3 presents representative photos showing aggregated yeast cells and hyphae observed in the plugs. Narrow (2.1-5.3 μm in width) and curved hyphae, sometimes branched and with lateral blastoconidia, were observed (Fig. 3a). Curved and thick-walled hyphae along with spherical thick-walled blastoconidia were abundant (Fig. 3a). Some hyphal cells formed buds in which cell widths at the terminus were larger than mid-cell (Fig. 3b), resembling the spore extrusion from hypha reported by Dorn and Roehnert.3 Multiple septum-like structures in the hyphal cells were clearly visible (Fig. 3c, yellow arrows).

In addition, the septum-like structures were observed in the cell terminus (Fig. 3c, yellow arrowheads), suggesting that this structure remained on the plane of division after the conidia emerged from the hyphae. Yeast cells showed globose and ovoid forms, with axes of 2.3–5.4 μm (minor axis) by 3.4–6.0 μm (major axis). Budding was usually monopolar through a broad base. Although some daughter cells were elongated, the base was not perfectly constricted so that they remained constant in size (Fig. 3d). Similar to the hyphal cells, septum-like structures in the yeast cells were also observed (Fig. 3c, yellow arrows). Seven species were detected: *Malassezia dermatis*, *Malassezia furfur*, *Malassezia globosa*, *Malassezia japonica*, *Malassezia restricta*, *Malassezia slooffiae*, and *Malassezia sympodialis*.
like structures were also observed in the terminus of yeast cells after cell division (Fig. 3e). These morphological characteristics are often described as an SM configuration that is mainly observed in skin scales of human pityriasis versicolor lesions. Therefore, we identified the yeast cells and hyphae in keratotic plugs as *Malassezia* species on the basis of this configuration.

*M. restricta* and *M. globosa* were identified in all samples in healthy individuals and patients with *Malassezia*-related skin diseases. However, their colonization level differed in each
host pathology. The level of M. restricta colonization was overwhelmingly dominant in patients with seborrhoeic dermatitis, whereas the level of M. globosa colonization was higher in patients with pityriasis versicolor.\(^2,3\) The typical SM morphology observed in lesions of patients with pityriasis versicolor is identical to what we found in keratotic plugs in this study. Of the genus Malassezia, only M. globosa and Malassezia furfur form hyphae.\(^4\) Of the 10 cases, hyphae were observed in 5, among which M. furfur was detected in 2; however, its relative abundance was less than 1%. From these findings, we conclude that the hyphae formed in the keratotic plugs were likely M. globosa. The morphology changes depended on environmental conditions. Because pityriasis versicolor develops mainly in the summer season, amino acids in sweat are considered one of the inducers. Glycine, which is abundant in sweat, induces hyphae \textit{in vitro}.\(^7\) Keratotic plugs are formed when sebum accumulates in the pores and blocks them to form comedos. Components of keratotic plugs consist of protein and sebum, including triglycerides and free fatty acids. However, it is unclear which component is involved in the hyphal induction of Malassezia species.

In conclusion, we demonstrated that Malassezia species show an SM morphology in healthy individuals and not only in lesions of patients with pityriasis versicolor. However, the number of samples in the study was limited. The study provides useful information to elucidate the mechanism of hyphal induction in Malassezia species.

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Conflicts of interest

The authors declare that they have no potential conflicts of interest to report.

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