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Case Report

Identification of *Trichophyton benhamiae* by MALDI-TOF Mass Spectrometry. First report in Peru

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ABSTRACT

Trichophyton benhamiae is an emerging zoophilic dermatophyte that mainly infects skin and scalp and has been reported in Asia, America and Europe. It has two phenotypic variants: one with white colonies and the other with yellow colonies. Morphological identification of *Trichophyton benhamiae* is not sufficient and can be confused with *Microsporum canis* or *Trichophyton mentagrophytes*. Genomic or proteomic analysis is required to establish a definitive identification. We present three dermatophyte strains obtained from the skin, nails and scalp of human infections in which macroscopic and microscopic examinations were not sufficient to identify the species. The final identification of *Trichophyton benhamiae* was performed using MALDI-TOF mass spectrometry. This is the first report of *Trichophyton benhamiae* in Peru.

1. Introduction

Trichophyton benhamiae is a zoophilic dermatophyte[1] that causes inflammatory fungal infections frequently affecting the skin and scalp.[2] It is considered an emerging mycosis[2,3] and its presence has been reported in Asia, America and Europe.[4–6] *T. benhamiae* infection is associated with contact with domestic animals such as guinea pigs, rabbits and dogs.[2] *T. benhamiae complex* includes six species: *T. benhamiae, T. bullosum, T. concentricum, T. erinacei, T. eriotrephon* and *T. verrucosum*. Among these, *T. benhamiae* has two phenotypic variants: one with white colonies (*T. benhamiaae var benhamiae*) and the other with yellow colonies (*T. benhamiae var luteum*)[7,8] which could be confused with *T. mentagrophytes* and *M. canis*, respectively.[3,9] The micromorphology shows hyaline septate hyphae, few to many pyriform microconidia on sessile or clustered arrangements; macroconidia and spiral hyphae may be few in white variants and absent in yellow variants. Morphological identification of *T. benhamiae* is not sufficient and species identification requires genomic or proteomic analysis.[5,7,8,10]

2. Cases

Between November 2021 and January 2022, from the mycological examination requests attended at the Roe Clinical Laboratory, Lima-Peru, we obtained three dermatophyte strains phenotypically identified as Trichophyton spp. The primary cultures were performed in tubes containing chloramphenicol sabouraud agar and mycosel agar. Subsequently (at 25°C), the cultures were reseeded on sabouraud agar and potato dextrose agar plates for macroscopic and microscopic examination (Figures 1 and 2).

VITEK®-MS instrument (bioMérieux, Marcy- l'Étoile, France) equipped with the VITEK®-MS IVD V4.0 database was used for final identification. The Fungal colonies were

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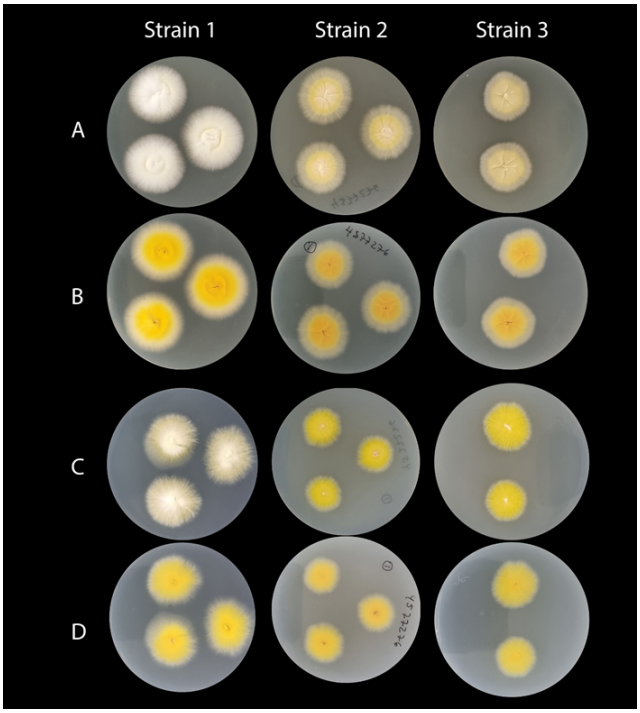

Figure 1: A): Front side colony on sabouraud agar, **B:** Reverse side colony on sabouraud agar, **C:** Front side colony on potato dextrose agar, **D:** Reverse side colony on potato dextrose agar

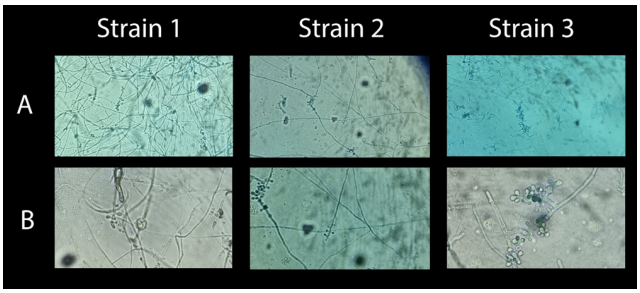

Figure 2: Conidia on potatod extrose agar. Lactophenol cotton blue stain. Strain 1 after 7 days, strain 2and 3 after at two weeks. **A**: hyphae, pyriform and globose microconidia; **B**: Chlamydospore, pyriform and globose microconidia, sessile arranged alternately on the hyphae as well as in clusters

applied on a VITEK® MS disposable target slide well followed by the application of formic acid and CHCA matrix (alpha-cyano-4-hydroxycinnamic acid).

2.1. Strain 1

A 57-year-old female patient with Hodking's lymphoma and skin lesions on the right forearm. A skin sample was collected and examined with KOH which revealed the presence of hyphae characteristic of dermatophytes.

After seven days of incubation on sabouraud agar, it developed a colony of 34 mm in diameter with a regular shape and defined edges. The front side of the colony showed a whitish, cottony color with a central concentric groove; the reverse side of the colony showed orange-yellow with a beige outer border. On potato dextrose agar, it developed a 32 mm diameter colony with an irregular shape and fuzzy edges. The front side of the colony showed a whitish central zone and a yellowish peripheral zone; the reverse side of the colony appeared orange-yellow with a beige outer border.

Microscopic examination revealed septate hyphae and many microconidia of variable size. The microconidia were pyriform and some were globose in appearance, sessile arranged alternately on the hyphae as well as in clusters. Macroconidia and spiral hyphae were not produced. Additionally, it showed some chlamydospores on potato dextrose agar.

Based on these morphological characteristics, the identification was established as *Trichophyton spp*.

2.2. Strain 2

A 27-year-old male patient with lesions suggestive of onychomycosis on the right first toe. A sample of the nail was collected and examined with KOH, but no fungal structures were detected. After seven days of incubation on sabouraud agar, it developed a colony of 27 mm in diameter with a regular shape and diffuse edges. The front side of the colony showed three concentric zones: beige interior, yellow middle and beige exterior, with radial grooves; the reverse side of the colony showed orange-yellow with a beige outer border. On potato dextrose agar, it developed a 21 mm diameter colony with a regular shape and fuzzy edges. The front side of the colony showed yellow coloration with a small beige external border; the reverse side of the colony showed orange-yellow with a small beige border.

Microscopy examination revealed septate hyphae without conidia. After two weeks on potato dextrose agar, the fungus developed a few microconidia of variable size. The microconidia were pyriform and some were globose in appearance; they were sessile arranged alternately on the hyphae as well as in clusters. Macroconidia and spiral hyphae were not produced.

Based on these morphological characteristics, the identification was established as *Trichophyton spp*.

2.3. Strain 3

A 2-year-old female patient presented with a scalp lesion. A sample of the scalp was collected and examined with KOH, but no fungal structures were detected. After seven days of incubation on sabouraud agar, it developed a colony of 26 mm in diameter with a regular shape and defined edges. The front side of the colony showed yellowish with a beige outer border and radial grooves; the reverse side of the colony showed orange-yellow with a small beige border. On

potato dextrose agar, it developed a 24 mm diameter colony with a regular shape and fuzzy edges. The front of the colony showed yellow coloration with a small beige external border; the reverse side of the colony showed orange-yellow with a beige outer crown.

Microscopy examination revealed septate hyphae without conidia. After two weeks on potato dextrose agar, the fungus developed a few microconidia of variable size. The microconidia were pyriform and some were globose in appearance; they were sessile and arranged alternately on the hyphae as well as in clusters. Macroconidia and spiral hyphae were not produced.

Based on these morphological characteristics, the identification was established as *Trichophyton spp*.

VITEK MS mass spectrometry identified the three strains as *T. benhamiae* with a confidence level of 99.9, 99.9 and 99.5 respectively (Figure 3).

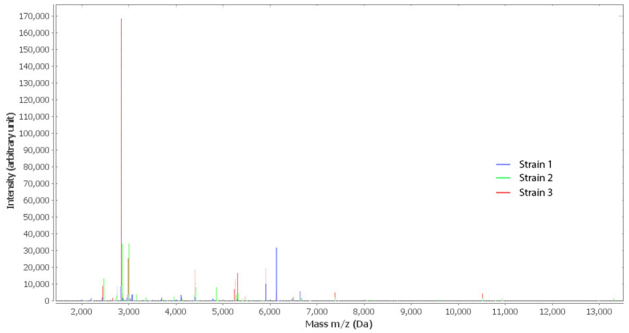

Figure 3: Overlap of mass spectral peaks. High coincidence was observed between strains 2 and 3 corresponding to the yellow phenotypic variant. (T. *benhamiae* var *luteum*)

3. Discussion

The morphological characteristics that we observed in the three strains align with the descriptions provided in previous studies: *T. benhamiae var benhamiae* (white colonies) showed a higher production of microconidia compared to *T. benhamiae var luteum* (yellow colonies)'[7] the microconidia had variable size and the presence of globose shapes.[2]

Previous studies have demonstrated that dermatophyte identification by Vitek MS mass spectrometry is highly accurate with respect to internal transcribed spacer sequencing (ITS).[11–13] The performance of different sample processing methods, including direct plate extraction and tube extraction with pretreatment, has been evaluated without finding significant differences between the two methods, associating the sensitivity and accuracy of the results to the database used.[14–16] According to previous studies, identification of *T. benhamiae* by mass spectrometry is accurate and reliable with respect to ITS sequencing.[2,10,17] The studies cited previously used the VITEK®-MS V2.0 to V3.2 databases; we used the V4.0 database and the direct plate extraction method.

In Peru, there are no updated reports of dermatophyte agents causing infection in humans; a review of 7185 cases between 1976-2005 reported the presence of *T. rubrum, T. mentagrophytes, T. tonsurans, M. canis, M. gypseum, E. floccosum* and *T. verrucosum*.[18] Two studies performed on Cavia porcellus breeding farms have reported the presence of *T. mentagrophytes* and *M. canis*.[19,20] The limited availability of tools such as mass spectrometry and molecular methods means that the identification of dermatophytes is mainly based on the recognition of macroscopic and microscopic characteristics of the colonies.

A limitation of our study is the lack of clinical and epidemiological data about contact with companion animals related to the transmission of this dermatophyte.

4. Conclusion

In conclusion, three dermatophyte isolates were identified as *T. benhamiae* by VITEK MS mass spectrometry, which represents the first report in Peru.

5. Source of Funding

None.

6. Conflict of Interest

None.

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