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First isolation of *Trichophyton bullosum* from a horse with dermatophytosis in Japan

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ABSTRACT

Trichophyton bullosum is a zoophilic dermatophyte that has been rarely isolated from horses and humans in Africa and Europe. This is the first reported isolation of *T. bullosum* from a horse with dermatophytosis in Japan. The isolate from a skin lesion formed a cream-colored and waxy colony that was slightly elevated in the center. Sequencing of the internal transcribe spacer region of the isolate revealed that it was 100% identical to that of *T. bullosum*.

1. Introduction

Equine dermatophytosis is a common skin disease of horses in Japan, where *Trichophyton equinum* and *Microsporum canis* (*M. equinum*) are the most common fungal pathogens [1]. Other reported causative agents of equine dermatophytosis include *Trichophyton mentagrophytes* and *Microsporum gypseum* [1,2].

Trichophyton bullosum is an almost-forgotten zoophilic dermatophyte that has been isolated in the past from the coat of horses in Africa (Tunisia and Mali (then French Sudan)) and the Middle East (Syria) [3,4]. In 2012, the first human infection was reported in Europe [3,4]; it was found in a rural area in France, where the dermatophyte was likely transmitted from a donkey with suspected dermatophyte lesions. Subsequently, it has also been isolated from a horse with dermatophytosis in the Czech Republic in 2013 [4]. However, there has been no report of the isolation of this species in Asia. To our knowledge, the present paper describes the first reported isolation of *T. bullosum* from a horse with dermatophytosis in Japan.

2. Case presentation

A female Japanese racehorse (2 years old) was referred to the Racehorse Hospital, Miho Training Center, Japan Racing Association, Japan, with a right forelimb laceration in 2020.

Physical examination revealed a circular erythema and hair loss on the left side of the neck; the affected area was 3 cm in size (Fig. 1a). Microscopic examination of skin scrapings from the lesion revealed dermatophyte arthroconidia around the hair (Fig. 1b). At this point, the horse was diagnosed as having dermatophytosis. The horse was treated with terbinafine ointment and cured after 2 months.

The skin scrapings from the left side of the neck were inoculated onto Sabouraud dextrose agar with 2×10^{-4}% nicotinic acid, and incubated at 28 °C. Within 21 days, a cream-colored and waxy colony that was slightly elevated in the center could be seen (Fig. 2a). Microscopic examination of the colony revealed the presence of intensely septated vegetative hyphae with an apparent fragmentation tendency (Fig. 2b). They included numerous thick-walled, intercalar and terminal chlamydospores, and were frequently in chains (Fig. 2b), indicating that the isolate was *T. bullosum* [4].

Genomic DNA samples were isolated as reported previously [5]. Molecular characteristics of the strains were also identified by sequence analysis of the internal transcribed spacer (ITS) region. The ITS region of the isolate was amplified using the universal fungal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGC) and ITS4 (5′-TCCTCCGCTTATTGATAGC) [6].

Thirty cycles of PCR amplification were performed with the following conditions: denaturation for 30 s at 95 °C, primer annealing for 30 s at 55 °C, and extension for 1 min at 72 °C in a total reaction

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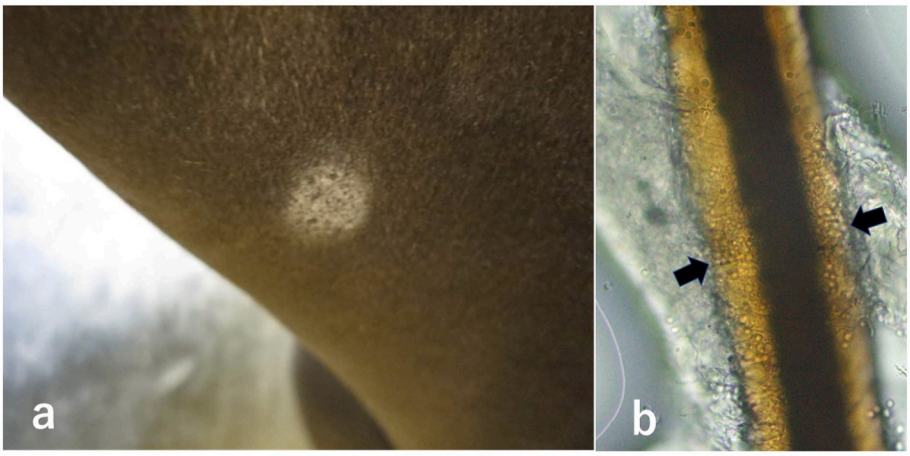

Fig. 1. a, The circular erythema and hair loss on the left side of the neck of the patient horse. b, Arthroconidia around the hair (Arrows).

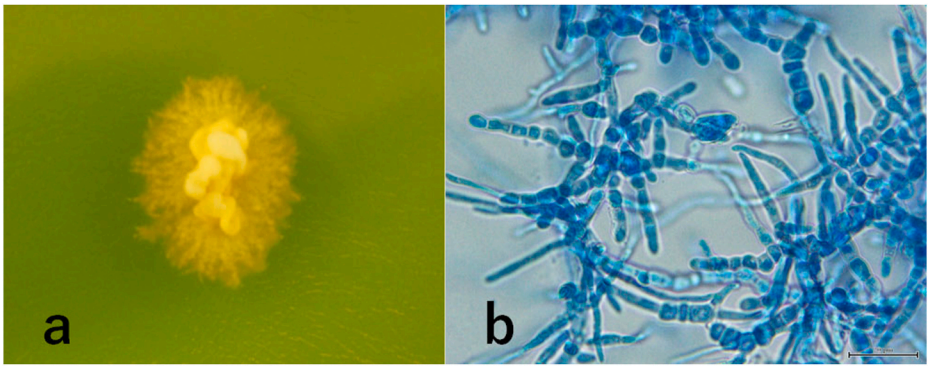

Fig. 2. Colony and microscopic morphologies of the isolate. a, Photo showing the cream-colored and waxy colony with a slight elevation in the center. b, Microscopic photo showing numerous thick-walled, intercalar and terminal chlamydospores that are frequently in chains.

volume of 30 μl of amplification mixture (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM deoxynucleotide triphosphate, 1.0 U *Taq* polymerase (Takara, Kyoto, Japan), and 50 μM of each primer). The resulting amplified DNA fragments were electrophoresed on a 2% (w/v) agarose gel with 1 × Tris-acetate-EDTA buffer, and visualized by ethidium bromide staining.

A DNA band of approximately 550 bp for each strain was excised from the gel, purified with the ExoSAP-IT® kit (USB Corporation, Cleveland, OH, USA), and sequenced on an ABI PRISM 3130 DNA Analyzer (Thermo Fisher Scientific, Inc., Tokyo, Japan).

Comparative nucleotide sequence analysis using the BLAST algorithm on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showed that the ITS sequence from the isolate was 100% identical to that of *T. bullosum* (*T. bullosum* genomic DNA containing ITS1, 5.8S rRNA gene, and ITS2, isolate CCF 4831, GenBank: LN589975.1).

The ITS sequences determined in this study have been deposited in the GenBank database (*T. bullosum* NUBS20002 genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, GenBank accession no. LC592175).

To analyze the mating type (*MAT*) gene loci of the isolate, PCR analysis of the alpha-box (*MAT1-2*) and high-mobility group (HMG; *MAT1-1*) genes was carried out using the genomic DNA from the isolate. The primers TmMATa1S and TmMATa1R amplified a 471-bp fragment of the *T. mentagrophytes* alpha-box (*MAT1-2*) gene. The primers TmHMG1S and TmHMG1R amplified a 524-bp fragment of the *T. mentagrophytes* HMG (*MAT1-1*) gene [5].

Genomic DNA sample (100 ng) from the clinical isolate was amplified by PCR in a volume of 30 μl using a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM of each deoxynucleoside triphosphate, 1.0 unit *Taq* polymerase (Takara), and 50 nM of the pair of primers. Amplification was carried out over 35 cycles consisting of template denaturation (1 min, 94 °C), primer annealing (1 min, 55 °C), and polymerization (2 min, 72 °C). The PCR products were detected after electrophoresis on 2% agarose gels by staining with ethidium bromide and visualization under ultraviolet light.

The HMG (*MAT1-1*) gene was detected in the isolate, whereas the alpha-box gene (*MAT1-2*) was not detected.

To investigate the nutritional requirements of the isolate, Trichophyton agar media 1, 3, 4, and 5 (Thermo Fisher Scientific, Waltham, MA, USA) were used to determine the inositol, thiamine, and nicotinic acid requirements [4,7].

The isolate grew well on Trichophyton agar 3, 4, and 5, and grew slightly on Trichophyton agar 1 and 2, indicating that inositol, thiamine, and nicotinic acid are required. Of interest, *Trichophyton verrucosum,* which is phenotypically similar, shows good growth on Trichophyton agar 3 and 4, indicating a requirement for inositol and thiamine [4,7].

3. Discussion

This is the first reported isolation of *T. bullosum* from a horse with dermatophytosis in Asia. *T. bullosum* is very rare in Europe [4]. Two cases of human dermatophytosis have been reported in France [3,8], but no samples were collected from the source animals in that country. The species has also been isolated from the hair coat of horses in Africa

(Tunisia and Mali) and the Middle East (Syria) [4]. The colonies are slow-growing, cream-colored, waxy, and slightly elevated in the center when grown on Sabouraud dextrose agar. The morphology and physiology of *T. bullosum* resemble those of *T. verrucosum,* and it might have been misidentified as *T. verrucosum* in the past [4]. However, the HMG (*MAT1-2*) gene was detected in the isolate, whereas the alpha-box (*MAT1-1*) gene was not detected. All isolates of *T. verrucosum* from the Czech Republic, Netherlands, United States, and Japan have been surveyed for the presence of the *MAT 1-2* allele [9,10]. It was also proved that both species are different in the *MAT* gene marker. The results suggested that *T. bullosum* and *T. verrucosum*, which are phenotypically similar, may have resulted from divergent evolution from a common ancestor.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone were responsible for the content and writing of this paper.

References

[1] Y. Kinoshita, Fungal Diseases of Horses, second ed., Japan Livestock Industry Association, Tokyo, 2016, 2016.
[2] S. Okoshi, A. Hasegawa, Animal mycoses. Japan, J. Med. Mycol. 9 (1968) 233–240.
[3] E. Sitterle, E. Frealle, F. Foulet, O. Cabaret, G. Cremer, J. Guillot, L. Delhaes, F. Botterel, *Trichophyton bullosum*: a new zoonotic dermatophyte species, Med. Mycol. 50 (2012) 305–309.
[4] P. Lyskova, V. Hubka, A. Petricakova, R. Dobias, A. Cmokova, M. Kolarik, Equine dermatophytosis due to *Trichophyton bullosum*, a poorly known zoophilic dermatophyte masquerading as *T. verrucosum*, Mycopathologia 180 (2015) 407–419.
[5] R. Kano, M. Kawasaki, T. Mochizuki, M. Hiruma, A. Hasegawa, Mating genes of the Trichophyton mentagrophytes complex, Mycopathologia 173 (2012) 103–112.
[6] H.P. Hinrikson, S.F. Hurst, T.J. Lott, D.W. Warnock, C.J. Morrison, Assessment of ribosomal large-subunit D1-D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as target for molecular identification of medically important *Aspergillus* species, J. Clin. Microbiol. 43 (2005) 2092–2103.
[7] D. Ellis, S. Davis, H. Alexiou, R. Handke, R. Bartley, Descriptions of Medical Fungi, second ed., Adelaide Medical Centre for Women and Children, Adelaide, 2007.
[8] M. Sabou, J. Denis, N. Boulanger, F. Forouzanfar, I. Glatz, D. Lipsker, P. Poirier, E. Candolfi, V. Letscher-Bru, Molecular identification of *Trichophyton benhamiae* in Strasbourg, France: a 9-year retrospective study, Med. Mycol. 56 (2018) 723–734.
[9] S. Kosanke, L. Hamann, C. Kupsch, S. Moreno Garcia, A. Chopra, Y. Gräser, Unequal distribution of the mating type (*MAT*) locus idiomorphs in dermatophyte species, Fungal Genet. Biol. 118 (2018) 45–53.
[10] R. Kano, E. Yoshida, T. Yaguchi, V. Hubka, K. Anzawa, T. Mochizuki, A. Hasegawa, H. Kamata, Mating type gene (*MAT1-2*) of *Trichophyton verrucosum*, Mycopathologia 177 (2014) 87–90.