

African Journal of Microbiology Research

Full Length Research Paper

# Identification of *Basidiobolus* species from the common house gecko (*Hemidactylus frenatus*) and their association with isolates from human basidiobolomycosis

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> > Received 18 January, 2021; Accepted 11 March, 2022

The genus *Basidiobolus* contains large groups of terrestrial fungi including the etiological agents of human gastrointestinal basidiobolomycosis (GIB). This study aimed to identify *Basidiobolus* species from the common house gecko and to compare them with human GIB isolates. Gecko and human GIB samples were collected from Muhayil Aseer area, south Saudi Arabia (2017-2019). Isolation of fungi from the gut contents of geckos was performed using Sabouraud dextrose agar incubated aerobically at 30°C for five days. Suspected *Basidiobolus* species were tentatively identified using routine bench tests and phenotypes were authenticated by phylogenetic analysis of the large subunit ribosomal RNA gene. Isolates (n = 10) were found to have zygomycete-like phenotypic characteristics. In the 28S ribosomal RNA gene phylogenetic tree, the strains assembled in the subclade encompass *Basidiobolus* spp. along with previously reported isolates from human' GIB. The strains had a close resemblance with *Basidiobolus haptosporus* (99.97%) as well as with *B. haptosporus* var. *minor* (99.97%). One isolate (L3) falls within the subclade containing *B. haptosporus* strain NRRL28635. The recovery of similar isolates from both humans and gecko lizards in one geographic region is an important development toward knowing risk factors for GIB.

Key words: Zygomycetes, Entomophthoromycota, Basidiobolus, lizards.

# INTRODUCTION

Basidiobolus species belong to the family Basidiobolaceae (phylum Entomophthoromycota) which includes a vast collection of terrestrial fungi that have been categorized in the phylum *Zygomycota* (Gryganskyi et al., 2013, 2012). Recently, *Zygomycota* was noticed as phylum particularly difficult to assess taxonomically and

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> phylogenetically and recent molecular and morphological studies clearly rejected the previously assumed monophyly of the *Zygomycota* (Gryganskyi et al., 2013; Möckel et al., 2022; Spatafora et al., 2016).

Basidiobolus can cause infections in animals as well as humans. This infection is also called entomophthoromycosis. It is characterized by its granulomatous nature and the formation of hard, nonulcerating subcutaneous masses on the limbs, chest, back. and buttocks. Systemic infection is rare. Basidiobolus is a true pathogen, causing infections in immunocompetent hosts. However, recent data on angioinvasive infections due to Basidiobolus in immunocompromised patients suggest that it is emerging as an opportunistic pathogen as well (Kohler et al., 2017; 2000). Ribes et al., Human infections due to Basidiobolus are reported mostly from Africa, South America, and tropical Asia. Basidiobolus ranarum is the etiologic agent of subcutaneous chronic zvgomvcosis in man. Cases of invasive zygomycotic infection caused by B. ranarum in young humans and their clinical and pathological features have been reviewed with highlight reported from different countries (Hussein et al., 2021; Nazir et al., 1997). This suggests that gene or genome duplication may be an important feature of B. ranarum evolution and suggests that B. ranarum may have mechanisms which favors their difference from other related taxa or possessing unique properties (Elya and De Fine Licht, 2021; Henk and Fisher, 2012).

The significance of exotic animals maintained in temperate regions is a potential carrier of pathogenic organisms such as Basidiobolus spp. (Feio et al., 1999; Maniania et al., 2008). Cutaneous lesions in amphibians, subcutaneous infection in horses, and gastrointestinal lesions in dogs have so far been reported (Carmo et al., 2020; Gugnani, 1999; Khan et al., 2001; Nazir et al., 1997). The digestive tracts of various amphibians and reptiles have been found to harbor members of the family Basidiobolaceae. Similarly, isolation of such fungi was successful from soil and litter, but most readily from the intestinal contents of reptiles, amphibians, and some warm-blooded animals (Chaturvedi et al., 1984; Speare and Thomas, 1985). Basidiobolus fungus was isolated from the dung of amphibians, reptiles, and insectivorous bats, as well as wood lice, plant debris, and soil. The habitat of Basidiobolus haptosporus is often related to the gamasid mite Leptogamasus obesus (de Aguiar et al., 1980). Human cases of entomophthoromycosis due to B. haptosporus were linked to surgical wounds (Sood et al., 1997), skin and subcutaneous tissue (Bittencourt et al., 1980; de Leon-Bojorge et al., 1988; Hung et al., 2020), and deep invasive mycosis (Dworzack et al., 1978). A gastrointestinal entomophthoromycosis case due to B. haptosporus was described (Anand et al., 2010; de Aguiar et al., 1980). Many cases of gastrointestinal zygomycosis caused by B. haptosporus could be expected as either misdiagnosed or passed undiagnosed.

In Florida (USA), the occurrence of the fungus in the digestive tracts of many types of toads has been documented (Nelson et al., 2002). The common isolation of *B. haptosporus* DNA from sources related to the gamasid mite *Leptogamasus obesus*, and from non-soil samples seems not to be in line with its presumed ecosystem as an ubiquitous saprophytic soil fungi. This leads to another working hypothesis that a second host species is present in the life cycle of *B. haptosporus* (Werner et al., 2012).

Gecko lizard (*Hemidactylus frenatus*) is classified in the family Gekkonidae (infra-order *Gekkota*). It is a native species in Southeast Asia and is habitually seen in several Asian countries including Saudi Arabia. It is called the Asian House Gecko, or simply, House Lizard. It has been indicated that the house gecko has a substantial role in the epidemiology of salmonellosis and has an effect on general public health (Jimenez et al., 2015). However, there is no data on its role in transmitting or acting as a source for fungal infections such as basidiobolomycosis.

This study aimed to detect *Basidiobolus* spp. in gecko lizards from an area endemic to GIB in Aseer, Saudi Arabia. This study attempts to identify and establish risk factors related to GIB disease.

#### MATERIALS AND METHODS

#### Samples collection and study location

The material analyzed was from the gut contents of four gecko lizards (*H. frenatus*) captured during 2017 to 2019 from the Muhayil Aseer (Asir) area (N 18° 33' 5.7492", E 42° 2' 57.7248") south Saudi Arabia. Ethical approval was granted by the Ethics Committee, College of Medicine, King Khalid University. A small portion of the intestinal contents was collected from each gecko lizard and placed in sterile containers and immediately transported to the laboratory for processing.

Basidiobolus spp. (n = 6) which have been isolated from human GIB and identified before (Bshabshe et al., 2020) were included in the study. The strains were isolated given the laboratory codes along with DSM codes (as shown in Table 1).

## Isolation of fungi

Sabouraud dextrose agar (SDA; Difco Inc.) was used for the original isolation of fungi and for subsequent sub-culturing. Inoculated plates were then incubated at 25°C for up to one week. The isolates recovered from infected tissues were examined macroscopically and microscopically. Small pieces of colonies (thickness, 2 mm; diameter, 2 mm) were placed on lactophenol cotton blue (2 mL phenol, 2 mL lactic acid, 4 mL glycerol, 2 mL  $H_2O$ ) on a clean microscopic slide and examined microscopically.

#### Phenotypic identification

Identification of *Basidiobolus* spp. was based on the key of O'Donnell (1979) with the following morphological characters as

Laboratory (DSM*) code	Source	Accession number
L1 (DSM107663)	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256649
L3 (DSM 05995)	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256652
L4	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256646
L4G	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256647
Doza	Human GIB, Aseer region, Saudi Arabia (2013)	MH254938
9-4	Human GIB, Aseer region, Saudi Arabia (2014)	MH256645
F15-1	Human GIB, Aseer region, Saudi Arabia (2017)	MH256650
F43-5	Human GIB, Aseer region, Saudi Arabia (2016)	MH256651
V81 (DSM06014)	Human GIB, Aseer region, Saudi Arabia (2017)	MH256648
85-5	Human GIB, Aseer region, Saudi Arabia (2019)	N/A

 Table 1. Basidiobolus species isolated from gecko lizard guts and from human gastrointestinal basidiobolomycosis used in the study.

\*DSM, Deutsche Sammlung von. Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, 38124 Braunschweig, Germany; GIB, gastrointestinal basidiobolomycosis.

primary for identification of the genus: production of zygospores with or without smooth walls retaining short paired protuberances known as "beaks", and apical globose primary conidia that were forcefully discharged from the conidiophores, usually still connected to parts of the conidiophore commonly referred to as "skirts".

#### **DNA** sequence-based identification

DNA amplification and sequencing service was done by Macrogen (Seoul, Korea). Briefly, the primers LR0R 5' Inc. (ACCCGCTGAACTTAAGC) 3' and LR7 5' (TACTACCACCAAGATCT) 3' (Vilgalys and Gonzalez, 1990) were used for amplification of the partial large subunit ribosomal RNA (LSU) region and analyzed using the ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). DNA sequences of the strains evaluated were aligned with other reference fungal sequences available in the GenBank database using BLAST, and the alignments were inspected visually. The gaps generated were treated as missing data. The fungal DNA sequences were analyzed phylogenetically by the neighbor-joining method (Saitou and Nei, 1987) using MEGA software (Kumar et al., 2018). Verification for internal branches was calculated by using 100 bootstrapped data sets.

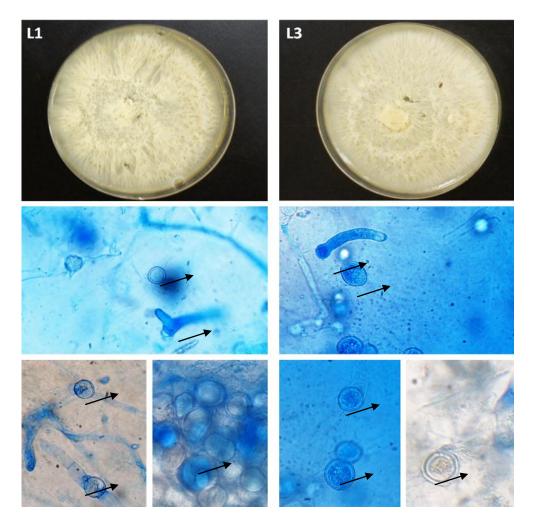
## Data availability

Data used to support the findings of this study are included within the article and its supporting information files. The 28S rRNA sequences established in this study and used for phylogenetic analysis have been deposited in the GenBank database ([https://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers shown in Table 1.

# RESULTS

Fungal isolates (n= 10) which have been isolated from the common house gecko as well as from human GIB between 2017 and 2019 were used for the analysis (Table 1). The organisms were fast growing with isotropic, pale colonies in the primary culture on SDA at 25°C. Within the first 3 days of incubation, flat membranous colonies with a smooth, glabrous, and waxy appearance developed. Older colonies (3 days old and older) became powdery in appearance, with short aerial mycelia, and developed radiating folds from the center of the colonies (Figure 1). Morphological characteristics conform to Basidiobolus species that are round, flat, waxy, glabrous, and radially folded colonies (Campbell et al., 2013; de Leon-Bojorge et al., 1988; O'Donnell, 1979). The tested strains were found to have phenotypic properties distinctive for members of the genus Basidiobolus. The 12 isolates from human cases of GIB and from geckos were identified as Basidiobolus spp. based on their macroscopic and microscopic features. Colonies were examined microscopically from day 3 to day 5 for aerobic SDA growth. Young colonies show colorless broad hyphae with few septa, with smooth, thick walls, and abundant large, spherical, darkly colored chlamydospores and zygospores are formed (Figure 1).

Comparison of the 28S rRNA sequences of the isolates corresponding nucleotide sequences with of representatives of the class Basidiobolomycetes confirmed that they belong to the genus Basidiobolus. Isolates L1, L4, and L4G were found to have identical 28S rRNA sequences. The strains formed a monophyletic clade in the 28S ribosomal RNA gene. They shared 99.971% similarity with B. haptosporus NRRL28635, 99.969% with B. haptosporus var. minor strain ATCC 16579, 99.925% with B. ranarum and 100% similarity with previously reported human isolates from cases of GIB (Figure 2). The high 28S rRNA gene sequence similarities to the representatives of the genus Basidiobolus (93.9 to 98.7%) showed by these isolates support their placement in this genus. Few nucleotide mismatches were found within the isolates. One isolate (L3) fell within the subclade encompassing B.



**Figure 1.** Colony morphology (upper row) and microscopic appearance (lower row) of some *Basidiobolus* strains. The pale membranous colonies with smooth, glabrous, waxy appearance which show radiating folds from the center of the colonies. Microscopically, the organisms show broad coenocytic hyphae with few septa together with abundant large, spherical, darkly colored chlamydospores and zygospores (Lactophenol Cotton Blue ×100).

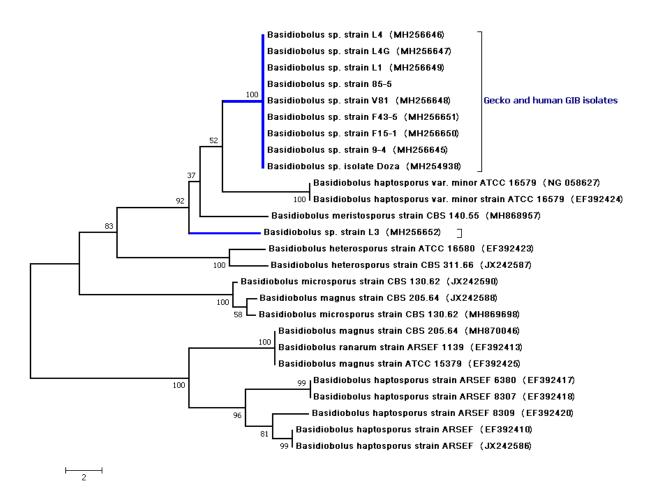
haptosporus strain NRRL28635. High-stringency BLAST (http://www.ncbi.nlm.nih.gov/) analysis of the three sequences showed a close similarity of human isolates to those from the lizards.

# DISCUSSION

The present study aimed to isolate and identify *Basidiobolus* fungi from the common house gecko. The study tried to link gecko as a potential risk of human GIB since house gecko is a contaminating agent for people in rural houses in Aseer region. This was done by comparing gecko isolates with previously reported human GIB isolates. The tested strains isolated from geckos have similar phenotypic properties distinctive for members of the genus *Basidiobolus* (Campbell et al., 2013; de Leon-Bojorge et al., 1988; O'Donnell, 1979).

The 10 isolates were identified as Basidiobolus spp. based on their macroscopic and microscopic features. Our initial phenotypic comparison of Basidiobolus spp. with those isolated from humans did not allow for solid conclusion on their close similarity, since growth and morphological traits alone are not always conclusive at the species level (O'Donnell, 1979). Application of DNA sequence analysis, using 28S rRNA gene was more decisive. This was useful in the present study supporting our hypothesis, as human and lizard isolates clustered in one clade related to but readily distinguishable from B. haptosporus and B. haptosporus var. minor (Figure 2). The results show that Basidiobolus isolates from humans and geckos from Aseer region have identical partial 28S distinguish rRNA sequences that them from representatives of closely related taxa, notably B. haptosporus and B. haptosporus var. minor.

The species of Basidiobolus have been identified, in



**Figure 2.** Neighbor-joining tree of aligned 28S large subunit ribosomal RNA genes of our strains from gecko lizards (L1, L3, L4, L4G) and five strains from humans (9-4, Doza, V81, F43-5, F15-1, 85-5) in relationship to closely related species. The numbers at the nodes indicate the levels of bootstrap support based on a neighbor-joining analysis of 100 resampled datasets. The bottom left bar represents 2 substitutions per nucleotide.

some records, as separate species including *B. ranarum*, B. meristosporus, and B. haptosporus, nevertheless, recent research on their antigens, restriction analysis of rDNA. and isoenzyme banding show that all Basidiobolus isolates that are pathogenic to humans be members of a single species, B. ranarum (Gugnani, 1999; Omar Takrouni et al., 2019). Similarly, antigenic analysis, isoenzyme banding and restriction enzyme analysis show that all human pathogens belong to B. ranarum. In two studies, B. ranarum was isolated in South India (Khan et al., 2001; Patro et al., 2019). Many articles have specified that the house gecko had a role in the epidemiology of salmonellosis and had an impact on public health (Jimenez et al., 2015). However, little evidence is available for its role in transmitting or representing a source for fungal infections, for example, basidiobolomycosis. A recent study showed that feces from South African reptiles often have Basidiobolus spp., indicating that they can add to the distribution of this fungus (Claussen and Schmidt, 2019).

B. haptosporus has been found in association with the

gamasid mite *Leptogamasus obesus* (Werner et al., 2012). Gastrointestinal entomophthoromycosis owing to *B. haptosporus* were rare (Werner et al., 2012) and found to be exposed by surgical wounds (Sood et al., 1997), having skin tissue linkage (Anand et al., 2010; Bittencourt et al., 1980; Hung et al., 2020), or systemic mycosis (van den Berk et al., 2006). It is obvious that many gastrointestinal zygomycetes caused by *B. haptosporus* are misdiagnosed or undiagnosed.

## Conclusion

Most of the isolates were identical to *B. haptosporus*-like fungi, including previous isolates from human GIB. The study suggests that the house gecko is a potential source of infection. The fungus is abundant in the lizard's guts and lizards live in close association with humans. Work is ongoing to further screen more lizard samples and their environmental habitat including water resources and soil in the GIB endemic area.

## **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

## ACKNOWLEGDEMENT

This work was supported by the Deanship for Scientific Research, King Khalid University (Project #GRP-28-1442).

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