



Article/Artigo

Evaluation of pathogenic fungi occurrence in traumatogenic structures of freshwater fish

Avaliação da ocorrência de fungos patogênicos em estruturas traumatogênicas de peixes fluviais

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ABSTRACT

Introduction: Fungal infections in human skin, such as sporotrichosis, can occur after fish induced trauma. This work aimed to identify fungi in freshwater fish that are pathogenic to humans. **Methods:** Extraction of dental arches from *Serrassalmus maculatus* (piranha) and *Hoplias malabaricus* (wolf fish), stings from *Pimelodus maculatus* (mandis catfish), dorsal fin rays from *Plagioscion* spp. (corvina) and *Tilapia* spp., for culture in Mycosel agar. Some cultures were submitted to DNA extraction for molecular identification by sequencing ITS-5.8S rDNA. **Results:** Cultures identified most yeast as *Candida* spp., while sequencing also permitted the identification of *Phoma* spp. and *Yarrowia lipolytica*. **Conclusions:** While the search for *S. schenckii* was negative, the presence of fungus of the genera *Phoma* and *Candida* revealed the pathogenic potential of this infection route. The genus *Phoma* is involved in certain forms of phaeohyphomycosis, a subcutaneous mycosis caused by dematiaceous fungi, with reports of infections in human organs and systems. Traumatizing structures of some freshwater fish present pathogenic fungi and this may be an important infection route that must be considered in some regions of Brazil, since there are a large number of a fisherman in constant contact with traumatogenic fish.

Keywords: Freshwater fish. Pathogenic fungi. *Phoma*. Human mycosis. *Candida* spp. Fishermen.

RESUMO

Introdução: Infecções fúngicas na pele humana (como a esporotricose) podem se manifestar após traumatismos por peixes. O objetivo deste trabalho é procurar fungos patogênicos para o homem em peixes fluviais. **Métodos:** Extração de arcadas dentárias *Serrassalmus maculatus* (piranha) e *Hoplias malabaricus* (traíra), ferrões de *Pimelodus maculatus* (mandis), raios da nadadeira dorsal de *Plagioscion* spp. (corvina) e *Tilapia* spp. para a realização do cultivo em agar Mycosel. Algumas culturas foram submetidas à extração de DNA para a identificação molecular pelo sequenciamento da região ITS-5.8S do rDNA. **Resultados:** As culturas mostraram que a maioria das leveduras era *Candida* spp. e o sequenciamento também permitiu a identificação de *Phoma* spp. e *Yarrowia lipolytica*. **Conclusões:** Embora a pesquisa para *S. schenckii* tenha sido negativa, a presença de fungos do gênero *Phoma* e *Candida* revela o potencial patogênico desta via de infecção. O gênero *Phoma* está envolvido em alguns casos de feohifomicoses, micoses subcutâneas causadas por fungos dematiáceos com relatos de infecções em órgãos e sistemas humanos. As estruturas traumatizantes de alguns peixes fluviais apresentam fungos patogênicos e esta pode ser uma importante via de infecção que deve ser considerada em algumas regiões do Brasil, uma vez que há um grande número de pescadores e peixes traumatogênicos.

Palavras-chaves: Peixes fluviais. Fungos patogênicos. *Phoma*. Micoses humanas. *Candida* spp. Pescadores.

INTRODUCTION

Fungi are ubiquitous and are found in soil, water, vegetables, animals and in humans. Fungal species undergo changes in incidence and according to location, season and air moisture content, as well as other variables¹. The growth of fungi can be divided into two phases, vegetative and reproductive. Most of these microorganisms are made up of microscopic filaments with well-defined cell walls called hyphae. Fungi were classified as plants until 1969 and then inserted into a separate kingdom called Fungi. In fact, they are phylogenetically more closely related to animals than plants and some of them are responsible for infections in several tissues. *Sporothrix schenckii* is an example of a fungus causing infections in humans; it causes subcutaneous mycosis with acute or chronic evolution. Fungi are installed by previous injuries, such as scratches by sharp plant or animal structures^{2,3}. There are well documented infections transmitted by dog bites⁴, rats⁵ and other animals. Sporotrichosis has also been documented from handling fish, originally reported by Mayorga & Caceres in 1978⁶ and more recently in Brazil^{7,8}. The incidence of accidents in river fishermen appears to be high, as observed in retrospective and prospective studies⁹. Poor working conditions and a lack of preventive measures are precipitating factors and the main fish species causing accidents are of commercial value and present traumatic structures or are venomous, such as mandis catfish, tilapia, traíras, piranhas and others. A large number of injuries and envenomings result in secondary fungal and bacterial infections that require further study to develop improved treatment and preventive measures against these accidents in fishing communities¹⁰. The objectives of this communication were to research the possible presence of pathogenic fungi in common fish present in the nets of fluvial fishermen.

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Received in 14/09/2010

Accepted in 24/11/2010

METHODS

Considering the cases of sporotrichosis reported by one of the authors^{7,8}, this work aimed to verify the existence of *S. schenkii* and other potentially pathogenic fungi in traumatogenic structures (stings, rays of fins and sharp teeth) of fish cited as the most hazardous by local fishermen and documented as such in previous publications⁹. The methods were applied in the same river where the cases of sporotrichosis were previously identified, in the Piracicaba River, near the Tietê River, in the central region of the State of São Paulo, Brazil. In three samples collected on three mornings of daily routine work of the colony of fishermen, two specimens each of corvina or freshwater silver croaker (*Plagioscion squamosissimus*), piranha (*Serrasalmus maculatus*), dog-fish (*Acestrothyichus lacustris*), mandijuba or mandiamarelo catfish (*Pimelodus maculatus*), tilapia (*Tilapia* spp.) and wolf fish (*Hoplias malabaricus*) were obtained.

A sterile scalpel blade was used to remove traumatic structures from each fish, which were then directly inoculated into culture medium: upper and lower dental arches from *Acestrothyichus lacustris*, *Hoplias malabaricus* and *Serrasalmus maculatus*; dorsal fin rays from *Plagioscion squamosissimus* and *Tilapia* spp. and stingers from

Pimelodus maculatus (**Figure 1**). These steps were conducted where the fish were captured, at the fishermen's colony on the Piracicaba River, close to the junction with the Tietê River.

The cultures were duplicated and incubated at 25°C and 35°C. Cultures at 25°C, close to ambient temperature, were used to investigate fungal flora and assess possible changes in fungi morphology. Cultures at 35°C were intended to investigate the possible presence of fungal pathogens, because this is closer to human body temperature and pathogenic fungi, such as *S. schenkii*, show increased tolerance to heat.

The yeasts were isolated in the samples and maintained in Sabouraud Dextrose Agar (SDA) with subcultures every 15 days. During the final period, the yeasts were cultivated on CHROMagar-Candida (CHROMagar-Candida, Difco™), a differential culture medium for identifying *Candida albicans*, *Candida tropicalis*, and *Candida* spp., by respectively developing green, blue, and pink colonies¹⁰. The CHROMagar-Candida plates were divided and, following colony cultivation, were incubated up to 25°C for a maximum of five days to observe staining¹⁰.

Ethical considerations

The study was approved by Botucatu Medical School Animal Research Ethics Committee.



FIGURE 1 - Left, a and b: mandis and stings (*Pimelodus maculatus*). Right, top: teeth from piranha (*Serrasalmus maculatus*) and traíra (*Hoplias malabaricus*). Right, below: fin rays of *Tilapia* spp.

RESULTS

Figure 2 is a macroscopic view of the isolated colonies. Evaluation by CHORMagar™ enabled most of the yeasts to be classified. One isolate obtained from *Tilapia* was classified as *C. tropicalis* (blue color; **Figure 2**).

DNA extraction permitted high concentrations to be obtained, making it necessary to dilute samples for use in PCR reactions. The PCR reactions were all successful, demonstrating amplicons of approximately 400-650bp, as shown in **Figure 3**. These amplicons

were purified and subjected to gene sequencing analysis to obtain the nucleotide sequence to compare with NCBI genomic databases (National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool for nucleotide).

Nucleotide sequence analyses presented consistency with fungi of the genus *Phoma* spp. and *Yarrowia lipolytica*, the latter is used in genetic engineering experiments and is currently reported as nonpathogenic, while the former is a pathogenic fungus associated with diseases in various organs and human systems, primarily the skin. *Phoma* belongs to the group of phaeohiphomyces¹¹⁻¹⁴.

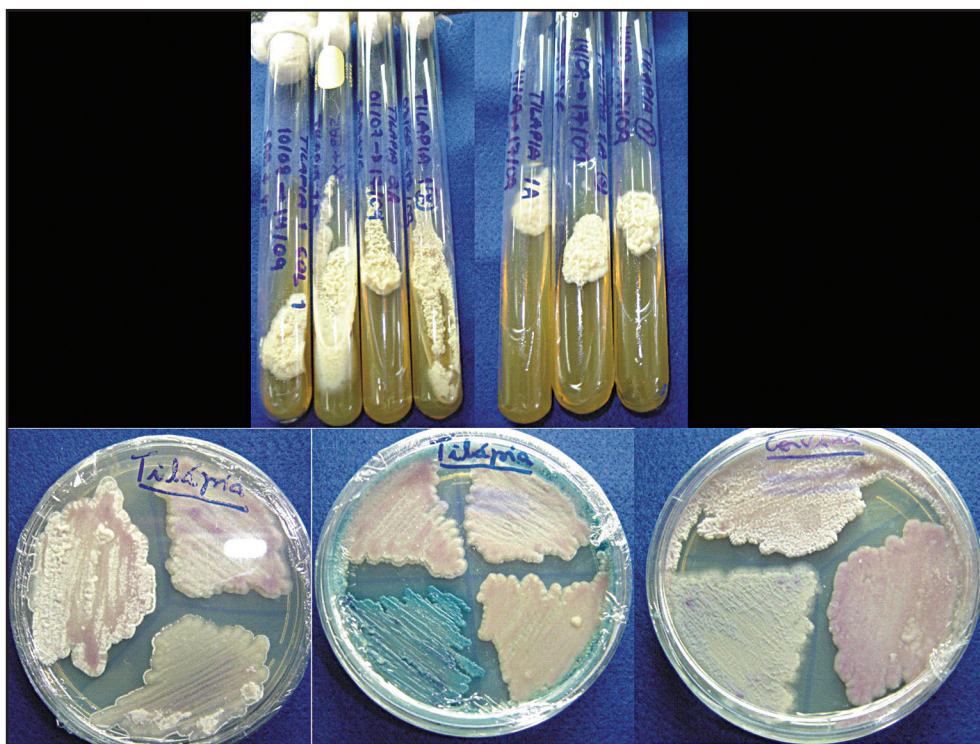


FIGURE 2 - Macroscopic view of yeast isolates on SDA agar at 25°C. Analysis of the color acquired in CHROMagar-*Candida*™ in yeast isolates grown at 25°C for five days.

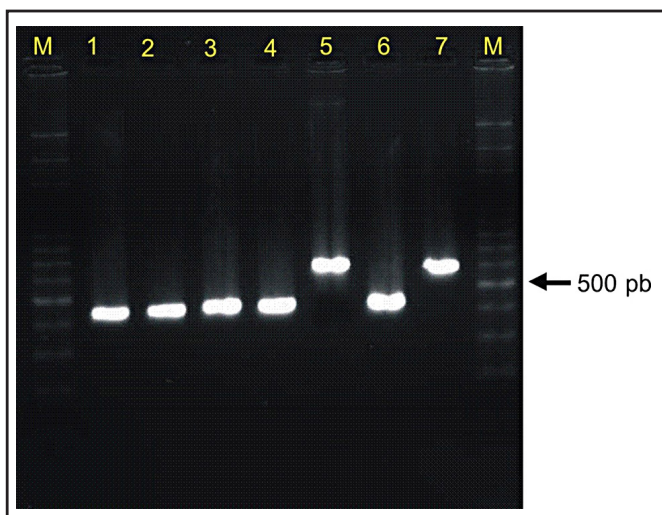


FIGURE 3 - PCR reaction for yeast isolates.

1: colony A from Mandi catfish number 1, 2: colony 2 from Mandi catfish number 2, 3: colony B from Mandi catfish number 2, 4: colony A from Corvina 1, 5: colony S from Corvina 1, 6: colony B from Corvina 1, 7: colony X from Corvina 1, M: 100bp Marker (LGC Biotechnology).

DISCUSSION

Due the fact that this research was original, certain difficulties were experienced when identifying the fungi, even with positive cultures from various yeasts and the inclusion of CHORMagar-*Candida* to analyze the colonies, the molecular methods were very important steps in helping to identify the microorganisms. While the association between sporotrichosis and trauma caused by fish has strong supporting evidence^{7,9}, the search for *S. schenkii* was unsuccessfully. However, the identification of fungi of the *Phoma* genus reveals the pathogenic potential of this infection route: this microorganism is

still poorly known in medical circles, even though several reports exists of human illness associated with this fungus¹¹⁻¹⁴, especially when iatrogenic states or immunosuppression is also present, or pathological states, such as AIDS and heterologous transplants.

In conclusion, the traumatic structures of certain river fish contain *Candida* yeasts and DNA extraction, PCR reactions and ribosomal region DNA sequencing revealed the presence of fungus of the *Phoma* genus and the species *Yarrowia lipolytica*. Further studies are required to improve current understand of these and other fungi, especially when considering the large number of fishermen and potentially traumatogenic fish in Brazil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Fábio Caetano Oliveira Leme and Marcos M. de B. Negreiros were awarded scientific initiation grants, PIBIC/CNPq (Scientific initiation scholarship recipient).

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