

# First Report of *Candida tropicalis* in Edible Freshwater Fish in the Philippines

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## ABSTRACT

In the course of establishing Free-Living-Amoeba (FLA) biodiversity from the intestines of edible freshwater fishes all over the Philippines, the presence of cyst-like structures in amoeba culture plates were observed, subjected to molecular identification and was later confirmed to be *Candida tropicalis* which is an emerging pathogenic yeast cell. This is the first report of a pathogenic yeast cell in an edible freshwater fish in the Philippines and is an important consideration as a possible source of fungal infection for humans, in particular individuals with immunocompromised status.

**Key words:** *Candida*, Fish, Freshwater, Lakes, Philippines, Yeast.

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## INTRODUCTION

*Candida tropicalis* is an emerging pathogenic yeast which can cause localized to systemic infections in both immunocompetent and immunocompromised individuals<sup>[1]</sup> while fishes have been identified as hosts of human protozoan pathogens like Free-Living Amoebae (FLA)<sup>[2,3]</sup> and *Cryptosporidium* spp.<sup>[4]</sup> to name a few. In our efforts to isolate FLA from edible freshwater fishes all over the Philippines, we came about the identification of a structure which was suspected to be an FLA cyst but was later confirmed to be *C. tropicalis* using molecular techniques.

We collected edible freshwater fishes from major lakes in the Philippines, the intestines of which were harvested aseptically and processed for FLA culture following the protocols from Milanez *et al.* 2017.<sup>[3]</sup> Upon the examination of culture plates on day three with 400X magnification using light microscopy, the presence of smooth and round cyst-like structures which can be suspected

as FLA cysts were observed. Since these structures did not fit morphologic descriptions for *Acanthamoeba* spp. Cysts,<sup>[5]</sup> confirmation thru polymerase chain reaction (PCR) using 18S universal eukaryotic primers EukA 5'AACCTGGTTGATCCTGCCAGT-3 and EukB 5'TGATCCTTCTGCAGGTTACCTAC-3 was performed following protocols from Medlin *et al.* 1988.<sup>[6]</sup> PCR amplicons were visualized in a 1.5% agarose gel stained with ethidium bromide and sent to a commercial sequencing company (Macrogen, Seoul, South Korea). Sequences were aligned using ClustalW of BioEdit with careful visual consideration of gaps and ambiguous sequences and were deposited to GenBank database. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7<sup>[7]</sup> with reference strains listed in Table 1.

We were able to isolate the cyst-like structure from edible freshwater fish *Glossogobius giuris* commonly known as tank goby and locally known as 'gunggong' in Lake Danao Sakanaw in the Eastern Visayas Island of the Philippines. Lake Danao Sakanaw is a popular tourist spot for picnics and recreational water activities. The cyst-like structure were round with smooth periphery and was between 5 to 10  $\mu$ m in size as shown in Figure 1. Molecular and phylogenetic analysis using reference strains as outlined in Table 1 revealed the cyst-like

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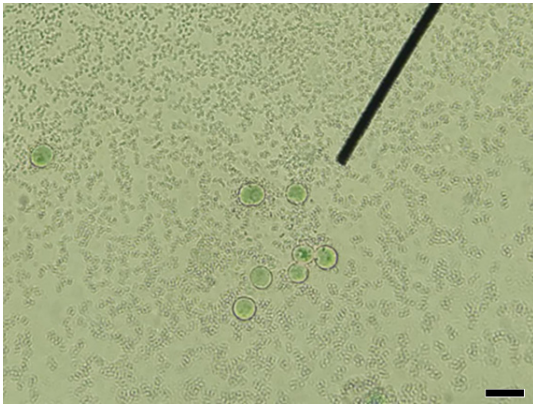


Figure 1: *Candida* yeast cells on non-nutrient agar surface 400X magnification, Scale bar = 10  $\mu$ m.

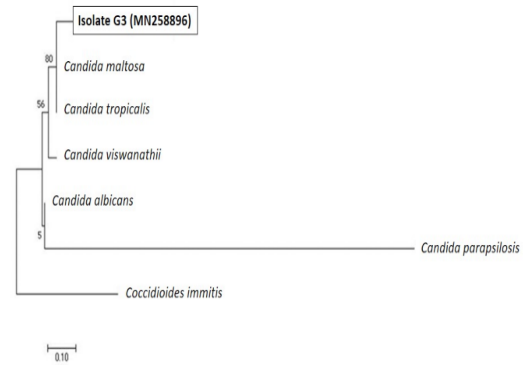


Figure 2: Maximum likelihood tree of isolate G3 *Glossogobius giuris* (in box). Tree was constructed using Kimura 2 parameter using MEGA 7 application. Phylogenetic analysis was carried out using 1000 bootstrap replicates.

Table 1: Reference strains of *Candida* spp. used for phylogenetic tree construction.

Organism	GenBank accession number	Source/location	References
<i>Candida tropicalis</i>	MN174079	none provided	Oraon and Saha, 2019 (unpublished)
<i>Candida parapsilosis</i>	BK010630	none provided	Oh et al. 2019
<i>Candida viswanathii</i>	MF339418	yeast strain	Vu et al. 2016
<i>Candida maltosa</i>	KY106554	yeast strain	Vu et al. 2016
<i>Candida albicans</i>	MN165403	none provided	Okolo, 2019 (unpublished)
<i>Coccidioides immitis</i>	AB232890	none provided	Sano et al. 2005 (unpublished)

isolate to be yeast cells of *C. tropicalis* with GenBank accession number (MN258896) as shown in Figure 2.

*C. tropicalis* is the most prevalent pathogenic yeast of the *Candida* non-albicans group and is increasing at a global scale owing to its growing resistance to fluconazole. Given this, researchers are still in the process of clarifying the fungi's pathogenic and virulence processes.<sup>[8]</sup> It seems unlikely that the *C. tropicalis* isolate is a normal microbial flora of the fish intestine. If that was the case then more of the yeast cells should have been identified from the culture plates, in particular during the early stages of incubation. It is more likely that the fish has ingested the yeast cells from its freshwater habitat and the role that the animal host in this instance remains unclear aside from being a potential reservoir for an emerging human pathogenic yeast species.

## CONCLUSION

Our results provide the first evidence of the presence of pathogenic fungal specie of *C. tropicalis* isolated from the intestine of an edible freshwater fish in the Philippines. This finding is significant in terms of humans in close contact with aquaculture resource, in particular freshwater fishes and freshwater fish pets. Caution should be taken in the handling of these aquaculture resource to avoid the potential transmission of not only fungal pathogens but other human pathogens as well. It will be of great benefit to avoid contact with aquatic resource and potentially contaminated water sources in cases of breaks on the skin and most especially if an individual has low or compromised immune status.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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