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Pichia guillermondi Wickerham and Burton, a New Yeast Record From India as a Fish Pathogen

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Abstract: A new yeast form, *Pichia guillermondi,* has been recorded as primary invader on the fish-- *Labeo rohita* and *Labeo calbasu* and identified by genomic DNA and ITS region sequence analysis. *Pichia guillermondi* has been reported, for the first time, from India as a fish pathogen. The climatic conditions have been found to affect the primary and secondary invaders on fish.

Keywords: *Pichia guillermondi, Labeo rohita, Labeo calbasu* Genomic DNA, ITS region

Introduction

In India, the incidence of disease has been observed in major carps cultured in different parts of the country (Gopalakrishnan, 1963, 1964). The adults, fries and fingerlings when transported over long distances get bruised on the body and, unless properly disinfected, these become sites of fungal infection resulting sometimes in large scale mortality. In certain other cases bacterial (Srivastava *et al.,* 2018) and yeast-like members have been found to be associated with the fishes that cause primary infection damaging the barriers of exoskeleton (the scales) and the mucus layer and prepare the site for secondary invasion by the water moulds to become the major causal organism.

Earlier records indicate that *Saccharomyces cerevisiae* has been reported on majority of fish specimens. During the present investigation, a new yeast form, *Pichia guillermondi,* has been recorded as primary invader on *Labeo rohita* fish and its molecular characterization has been done by ITS region sequence.

Materials and Methods

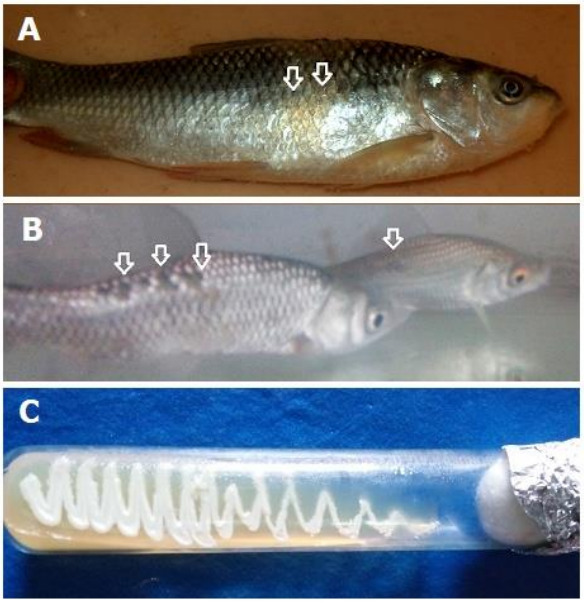
Few fish specimens of *Labeo rohita* and *Labeo calbasu*, collected from Ramgarh Lake,

Gorakhpur, yielded yeast forms on isolation, but no water mould was isolated. The host fishes were identified using the keys provided by Srivastava (2010). In some specimens yeast and water moulds were isolated simultaneously indicating that the yeast invaders were the primary ones and gave way to the secondary invaders – the water moulds. The culture was maintained on nutrient broth for further study.

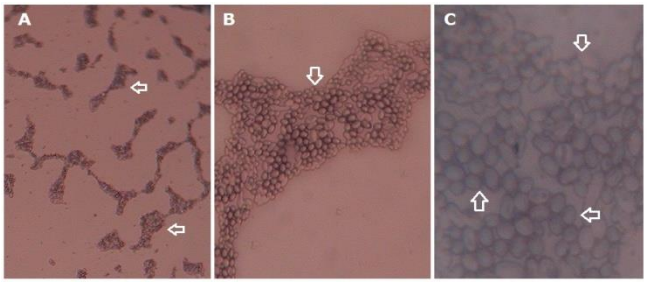
For ITS region sequence, the culture sample had been sent to Molecular Biology Services, First BASE Laboratories, Selangor, Malaysia and the results were obtained.

Results and Discussion

The specimens of *Labeo rohita* and *Labeo calbasu,* collected on August 12, 2016 from Ramgarh Lake, Gorakhpur; showed the presence of dull spots on their body surface probably due to the occurrence of certain yeast-like microbes (Figs. 1 A, 1B) – the primary invaders. Certain yeast-like fungal forms, which have been isolated from the primary infection sites have been cultured (Figs. 1C; 2 A-C) which required molecular characterization to have a correct identity (Figs. 3, 4).



Figs.: 1 A-C: Yeast-like fungal spots on the body of *Labeo calbasu* and *Labeo rohita* showing primary invasion. A: *Labeo calbasu* showing spot; B: *Labeo rohita* showing spots; C: Culture of *Pichia guillermondi* on nutrient agar slant.



Figs. 2 A – C: The yeast-like microbial sample isolated from the primary lesions on *Labeo rohita* and *Labeo calbasu.* A: The photomicrograph of yeast-like microbial sample. X 100; B: Same enlarged. X 400; C: Same enlarged. X 1000

Molecular characterization:

The genomic DNA of the yeast like fungal sample has been shown in Figs. 3 A and 3 B. Internal transcribed spacer (ITS) region, ~700bp have done. The Phylogeny analysis has been done using NCBI BLAST (Figs. 4, 5).

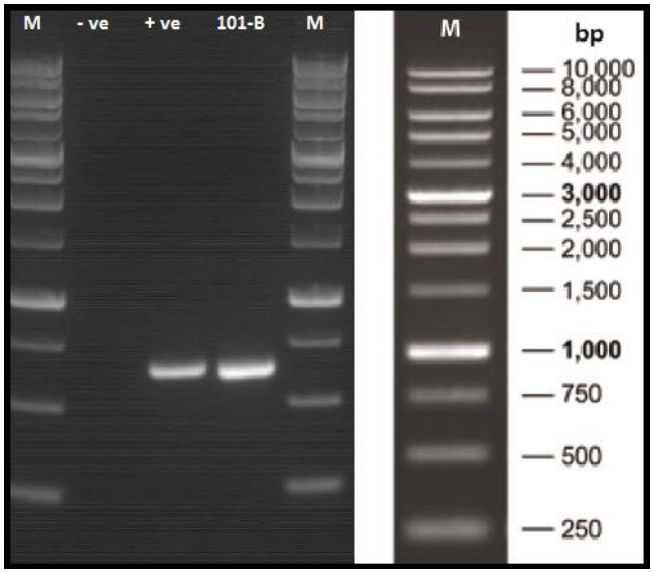


Fig. 3A: Genomic DNA of yeast-like microbial sample. -ve: PCR no-template control (water to replace DNA template); +ve Positive control (DNA extracted from *Candida hellenica used as template)*

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 TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGCG 60
CGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCT 120
AGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCA 180
AATTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAA 240
GAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTT 300
TGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTC 360
TCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGA 420
AAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTAT 480
CCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAA 540
CCAAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAA 600
GCGGAGGA                                                      608
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Fig. 3B: MG_BMBC/ 101-B, 608 bp

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	Meyerozyma guilliermondii isolate 2cpe-1 small subunit ribosomal RNA gene, partial sequence; int	1097	1097	100%	0.0	100%	KY401420.1
☑	Meyerozyma guilliermondii culture-collection CBS:12037 small subunit ribosomal RNA gene, partia	1097	1097	100%	0.0	100%	KY104257.1
☑	Meyerozyma guilliermondii culture-collection CBS:2084 small subunit ribosomal RNA gene, partial	1097	1097	100%	0.0	100%	KY104247.1
☑	Meyerozyma guilliermondii strain AP.MSU5 18S ribosomal RNA gene, partial sequence; internal tra	1097	1097	100%	0.0	100%	KT282394.1
☑	Meyerozyma guilliermondii isolate LMICRO181 18S ribosomal RNA gene, partial sequence; interna	1097	1097	100%	0.0	100%	KJ451705.1
☑	Meyerozyma guilliermondii strain AY17 18S ribosomal RNA gene, partial sequence; internal transc	1097	1097	100%	0.0	100%	KJ754141.1
☑	Meyerozyma sp. MM 20_T 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	1097	1097	100%	0.0	100%	KJ361493.1
☑	Meyerozyma guilliermondii isolate WM10.14 18S ribosomal RNA gene, partial sequence; internal t	1097	1097	100%	0.0	100%	JN183444.1
☑	Pichia guilliermondii strain HJM 18S ribosomal RNA gene, partial sequence; internal transcribed sp	1097	1097	100%	0.0	100%	EF191048.1
☑	Pichia guilliermondii isolate JHSd 18S ribosomal RNA gene, partial sequence; internal transcribed	1097	1097	100%	0.0	100%	DQ663478.1

https://www.ncbi.nlm.nih.gov/nuccore/KY401420.1,KY104257.1,KY104247.1,KT282394.1,KJ451705.1,KJ754141.1,KJ361493.1, JN183444.1,EF191048.1,DQ663478.1

Fig. 4: Top 10 Hits Blast Results against NCBI Nucleotide Collection (nr/nt) Database.

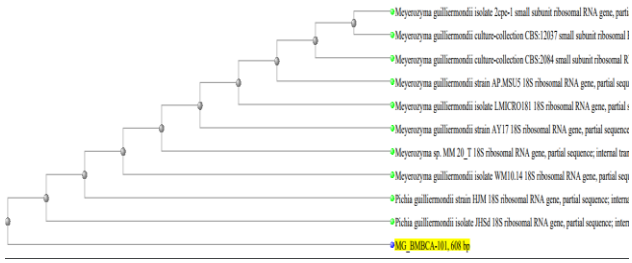

Fig. 5: Phylogenetic Tree – Neighbour Joining (Tree) by NCBI Blast Tree method.

The present sample, on the basis of ITS region sequence BLAST analysis, is allied to all the strains of *Meyerozyma guilliermondi* and *Pichia guilliermondi* strain (Accession DQ 663478.1). This has an indication that *Meyerozyma guilliermondi* and *Pichia guilliermondi* are synonyms. However, the phylogeny clearly indicates its direct alliance to *Pichia guilliermondi* strain (Accession DQ 663478.1) and therefore, the present isolate has been identified as a variant of *Pichia guilliermondi* strain (Accession DQ 663478.1).

The climatic conditions directly affect the pathogenesis in fish (Srivastava *et al.,* 2016). The present investigation also supports this contention when the bacterial/yeast pathogens develop primary lesions in monsoon season (July to September) when water mould concentration is supposed to be

less and in winter season (December to February) the water moulds have highest concentration (Prabhuji, 1979) and attack the primary lesions as secondary invaders.

Acknowledgement

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