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FIRST REPORT OF DIMORPHISM IN *ASPERGILLUS VERSICOLOR* ISOLATED FROM FRESH WATER FISH *HETEROPNEUSTES FOSSILIS*

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Abstract

The morphologic conversion of the dimorphic fungi from mold to yeast is required for virulence. Conversion from mold to yeast form may offer protection against killing by neutrophils, monocytes and macrophages. *Aspergillus versicolor* is a cosmopolitan fungus which rarely causes deep infections in humans. It is an occasional agent of onychomycosis. Here we report the dimorphism in *Aspergillus versicolor* isolated from skin of *Heteropneustes fossilis*. Cultures of *A. versicolor* were grown on SDA (Sabouraud Dextrose Agar) at 25^0 and 37^0 for 15 days. The pathogen grew well at both the temperatures. Micro morphology at 25^0 C exhibited normal branching of the hyphae along with formation of conidiophores and conidia. But after 10 days at 37^0C, hyphae began to convert into chains of conidia and up to 15^{th} day well defined yeast like capsules were found. Yeast like budding was also seen. This study suggests that *Aspergillus versicolor* itself could be a potential pathogen of warm blooded animals because it possesses dimorphism and has the ability to grow well at 37ºC.

Keywords: Dimorphism, Fungi, Heat shock proteins, Thermotolerance

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Introduction

The phase transition of the dimorphic fungi from mycelial to yeast form is an essential requirement for virulence. Conversion of mold to the yeast form may offer protection against killing by neutrophils, monocytes and macrophages. The ability to thrive at 37^0C is the characteristic of all human pathogens and has long been suspected to play a role in the pathogenesis of aspergillosis (Bhabhra and Askew, 2005). *Aspergillus versicolor* is a slow-growing filamentous fungus commonly found in damp indoor environments and on food products. Although it grows optimally between 22 and 26°C but it can tolerate at a larger temperature range from 4–40° C (Engelhart et al., 2002). Like other species of *Aspergillus*, *A. versicolor* is also an eye, nose, and throat irritant and is a major producer of the hepatotoxic and carcinogenic mycotoxin sterigmatocystin. It rarely causes deep infections in humans but it is an occasional agent of onychomycosis.

Temperature dependent phase transition is a characteristic feature of primary fungal pathogens. This morphologic conversion is needed for disease establishment in warm blooded hosts. The capability to grow at the upper limit of temperature for all eukaryotes is a unique stress resistant mechanism shown by a pathogen. Generally, morphogenesis from an environmental mould morphotype to a pathogenic yeast morphotype triggered by host conditions particularly 37^0C body temperature and leads to adaptations required to survive inside the host (Klein and Tebbets, 2007). Moreover, this differentiation is easily induced under laboratory conditions and is reversible. In the present study we describe temperature induced phase transition (from mould form to yeast form) in *A. versicolor* isolated from skin of fresh water fish *Heteropneustus fossilis*.

METHODOLOGY

Direct Microscopy

Direct Microscopy of infected region of skin was performed using lactophenol cotton blue.

Isolation of fungus

The infected *Heteropneustes fossilis* were collected from different ghats of river Ganges in district Varanasi, Uttar Pradesh India. The gross lesions on the skin and the scales were removed aseptically and their homogenate was prepared in 10 ml of sterile saline. This homogenate was further utilized for culturing the infecting micro-organism in SDA (Sabouraud Dextrose Agar) and Czapek-Dox media. The culture plates were incubated at 25 ºC for a period of 4 days.

Effect of temperature

For thermotolerance studies, culture plates were kept at 25ºC and 37ºC simultaneously for a period of 10 and 15 days.

RESULTS

Direct microscopy of skin of *Heteropneustes fossilis* showed long and septate hyphae and conidiophores which terminate in biseriate and small vesicles. Initially colonies appear typically white on SDA at 25ºC and change to yellow as they mature (Fig 1). On the basis of morphological characteristics of the colony grown on Czapek-Dox agar, the mould was identified as *Aspergillus versicolor*.

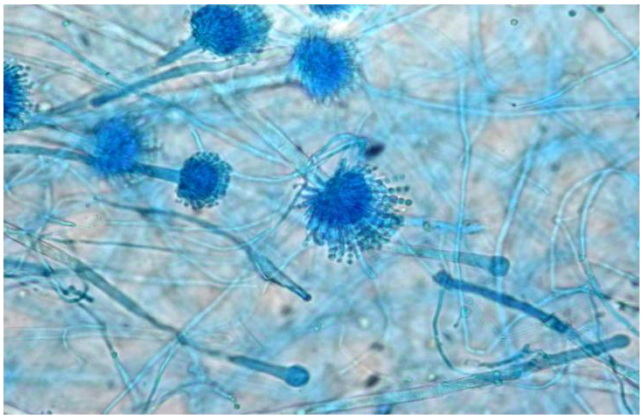

Fig 1: Direct microscopy of *A. versicolor* (1000 x Cotton blue)

Effect of Temperature:

Culture of *Aspergillus versicolor* was grown on SDA at 25^0 and 37^0C for 15 days. The pathogen grew well at 25^0 and 37^0C. Micromorphology at 25^0 C exhibited normal branching of the hyphae along with formation of conidiophores and conidia (Fig 1). But, at 37^0C after 10 days, hyphae began to convert into chains of conidia (Fig 2) and up to 15^{th} day well defined yeast like capsules were observed (Fig 3). Yeast like budding was also seen on 15^{th} day at 37º C (Fig 4).

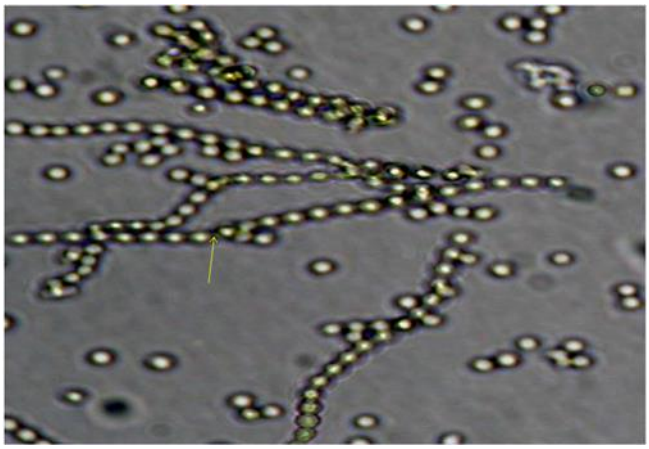

Fig 2: *A. versicolor* after 10 days at 37º C showing chains of conidia (400 x Lactophenol)

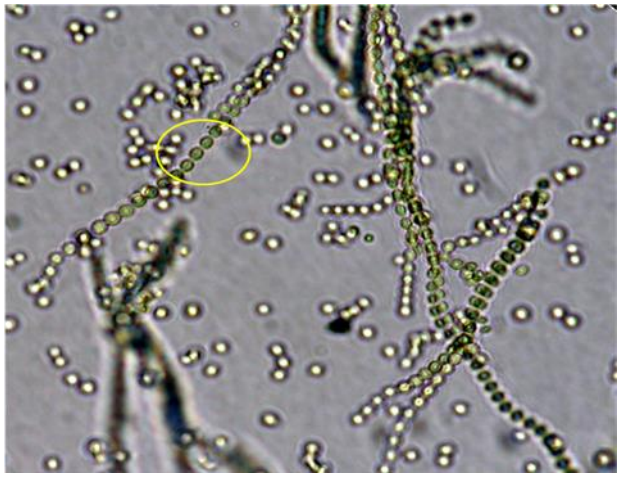

Fig 3: *A. versicolor* (15^{th} day) at 37º C showing yeast like capsules (400 x Lactophenol)

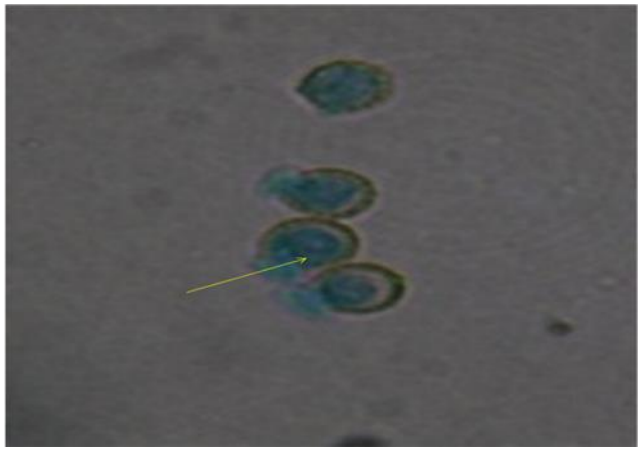

Fig 4: Arrow showing yeast like budding (1000 x Cotton blue)

DISCUSSION

Thermotolerance is directly related to the dimorphic behaviour of the organism. About 30 years ago, it was found that temperature-induced phase transition is an important virulence factor of systemic dimorphic ascomycetes (Klein and Tebbets, 2007). In general, dimorphic fungi grow in the form of mold at 22 to 25^0 C whereas they undergo transition to the yeast phase at 37^0 C. A lot of work is being done on the dimorphic organisms to determine the reason behind their successful transition from one form to the other. In 1989, Moresca and Kobayashi described several factors which directly or indirectly influencing dimorphism in *Histoplasma capsulatum* and suggested that optimum temperature for phase transition in a given strain is directly dependent on genetic determinant that are strictly correlated with temperature (Moresca and Kobayashi, 1989, Caruso et al., 1987, Medoff et al., 1987). Moreover, in *H. capsulatum*, it was found that during hyphal to yeast cell transition, the early morphological

changes are seen only at 18 to 20 hours, after that hyphal cells become enlarged and form chains of yeast like cells (Maresca and Kobayashi, 1989, Caruso et al., 1987, Medoff et al., 1987). Sometimes the yeast cells are also formed by the budding of unswollen cells. Similarly, in *Blastomyces dermatidis* the hyphal cells become yeast cells by a series of transformations (Howard and Herndon, 1960; Miyaji and Nishimura, 1977) and after 48 to 72 hours at 37^0C the cells (which are formed in alternate hyphal cells) broke apart releasing single cells that reproduce by budding but in present study the early morphological changes were observed at 10^{th} day after the shift to 37^0 C. The hyphal cells of saprophytic mycelia swelled and assumed yeast like shape. After 15^{th} day at 37^0 C, yeast like cells eventually fragmented to form chains of which ultimately gets separated from each other and reproduced by yeast like budding. Later stages of morphological changes in *A.versicolor* were visible after 15^{th} day which were quite similar to the odial cells of *B.dermatidis* formed after 48 to 72 hours at 37^0 C (Miyaji and Nishimura, 1977).

It has long been known that the event of morphogenesis from mycelia to yeast form leads to the adaptations needed for the survival of pathogen inside the host environment; formation of heat shock protein is one of them. A study was focused on determining the effect of temperature on synthesis of the heat shock proteins in a number of fungi such as *Rhizopus stolonifer, R. microsporous, Rhizomucor pusillus, Rhizomucor miehei, Fusarium* sps etc. depicting that there are different optimum temperatures for triggering the production of heat shock proteins for each organism. Studies on *A. fumigatus* suggest that a gene THTA is responsible for its thermotolerant behaviour. Another study in *Penicillium marneffei*, a very important dimorphic fungus suggests that the heat shock protein 70 (HSP70) gets up regulated on transformation from the mycelia to the yeast phase (Vanittanakom et al., 2010). Similar kind of study can also be performed in case of *A.versicolor* thus revealing the reason of its dimorphic behaviour.

From this preliminary study, we conclude that *A. versicolor* could be a potential pathogen of warm blooded animals because it possesses dimorphism and has the ability to grow well at 37°C. Further experimentation and molecular characterization are required to confirm our results.

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