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Morphological and Molecular Study of *Aspergillus tamarii* Kita

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Abstract

The scientist from Biology and related streams are better known to the importance of fungi, in which *Aspergillus* as most dominant, omnipresent, versatile genera with 132 species and 18 varieties are known till date. This number is increases day by day which create complexity and instability in their taxonomy. Traditionally mycologist generally uses morphological characters with the addition of physiological characters such as metabolites for systematic classification and identification. Due to continuous modification caused by Hybridization, Mutation, Recombination new species are arising. To avoid such confusion of new species, each species should be classified not only morphological and physiological characters but also with the addition of genomics and proteomics study, which enables us to study molecular biology of particular species.
In current study we attempt to elaborate the *Aspergillus tamarii* by SEM study and ITS r DNA sequencing.

Keywords: *A. tamarii,* morphology, *SEM, ITS, rDNA.*

INTRODUCTION

Aspergillus is most dominant omnipresent micro fungi. Researcher of every field has special attention on this particular organism due to its broad interdisciplinary scope. Many of the *Aspergillus species* are known due to adverse effects on animal and plants due to secretion of toxins. They are better known allergic or causing respiratory disease such as aspergillosis as they are opportunistic pathogen. The above mentioned use of *Aspergillus* is one of the most economically important genera of micro fungi, so the rigor and stability of its taxonomy is of significant practical concern (Geiser *et al*. 2007).

Fungi identification is difficult due to its variability of morphological (Different spores or reproducing bodies) and physiological (Metabolites) characters. The molecular identification based on Multilocus DNA sequence found to be good but it also has some loop holes for drawing phylogenetic relationship (Geiser *et. al*. 2007) where as Internal Transcribed Spacer (ITS) region plays the most promising role for the species identification (Park *et al*. 2007; Litaker *et al*. 2007) and it termed as a universal DNA barcode marker for fungi (Conrad *et al.* 2012). ITS were used for the identification of various fungi species and fungal groups (Druzhinina *et al.* 2005; Schwarz *et al*. 2006; Desnos-Ollivier *et al.* 2006; Summerbell *et al.* 2007; Geiser *et al.* 2004) . Other than ITS cox1, mt.DNA, intronic and exonic region were also used for identification and classification of some *Penicillium* and *Aspergillus* species (Seifert *et al*. 2007; Varga *et al.* 1993; Hamari *et al*. 1997; Kevei *et al.* 1996; Hamari *et al*. 2001; Juhasz *et al*. 2007).

MATERIAL AND METHODS

Morphological study – *Aspergillus tamarii* species was isolated from air using Petri-plate method and cultures were incubated for 5-7 days at 25^0C. Species were identified by microscopic observation of lectophenol stained of *Aspergillus tamari* and by comparing with available literature (Nagmani *et al.* 2006; Raper & Fennell 1965; Gilman 1945). Sub-cultures were obtained by using Czapek's Dox Agar medium. Little of the culture were dehydrated using H_2SO_4 as a dehydrant for SEM photography. Scanning Electron Microscopy of above specimen with different magnification was done by using SEM JEOL-6380A model at Department of Materials and Metallurgy, Vishsveswarayya National Institute of Technology, Nagpur.

Molecular study – Genomic DNA was isolated in pure form, from the above culture. Nearly 480 bpr DNA fragments were successfully amplified using fungal universal primers ITS4 & ITS5. The sequencing PCR was set up with ABI-Big Dye^R Terminator v3.1 Cycle sequencing kit. The raw sequence obtained from ABI 3100 automated DNA sequence was manually edited for inconsistency. The sequence data was aligned with publicly available sequences & analyzed to reach identity (Altschul *et al.* 1990). The molecular sequencing was done at NFFCCI- Agharkar Research Institute, Pune.

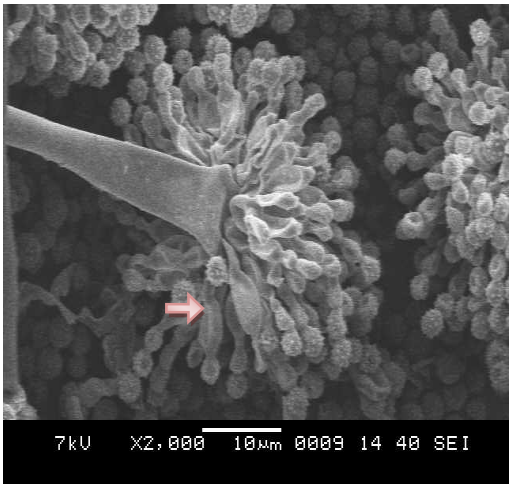
RESULTS AND DISCUSSION

Morphology of *Aspergillus tamarii* Kita

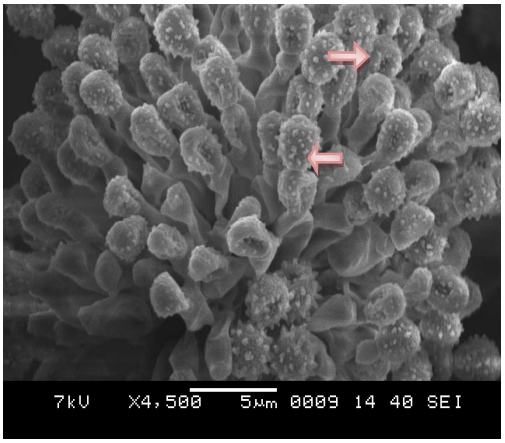
Colonies growing rapidly on CDA medium, mycelium white, floccose; Reverse uncolor, occasionally pinkish; of similar appearance to those of *Aspergillus flavus*, but with distinctly olive brown conidial color, conidia have thick, rough walls, more or less pyriform to globose; Phialides are uniseriate in small heads and Biseriate in large heads (Raper & Fennell 1965).

SEM photographs Shows the peculiar characters of *A. tamarii;* Stalk arising from submerged hyphae, up to 1-2 mm in length and 10-15 µm in diameter, the diameter is increasing towards apex and passing rather abruptly into vesicles. Vesicles vary greatly in size from 25-50 µm. Heads more or less columnar to globose up to 350 µm in diameter, with radiating chains and columns of conidia (Catenate arrangement). Acropetal conidia grow on uniseriate phialides with size 7-10 X 3µm. Conidia more or less pyriform to globose, 5-8 µm in diameter. The surface ornamentation of conidia has individual spines that are larger and more sharply define and pores were seen on equatorial junctions (resembling with walnut shells). The same characters were identified by previous workers (Yoko *et al*. 1999; Thaware *et al.* 2011; Thaware 2014).

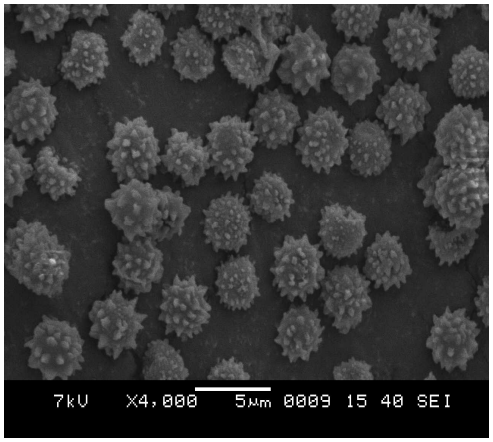
I.SEM photograph of *A.tamarii* having catenate conidia.



II.SEM photograph of *A.tamarii* having Uniseriate Phialides



III. SEM Photograph of *A.tamarii* having acropetal conidial chains with pores on conidia.



IV. Rough Thorny conidia.

Figure 1:

Molecular study of *Aspergillus tamarii*

The tested *Aspergillus* isolate showed 98% sequence similarity with *Aspergillus tamarii.*

Sequence analysis with NCBI accession number KJ013529.1 *Aspergillus tamarii* strain LS 01 resulted in following alignment statistics.

Alignment statistics: Query Length-480, Score- 830 bits (449), Expect- 0.0, Identities-469/480 (98%), Gaps-6/480 (1%), Strand- Plus/Minus.

```
Query 1        GCCTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 470      GCCTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTG  409

Query 61       CCTTTGGGGCCCGTCCCCCCCGAAGAGGGGACGAC---CCAACACACAAGCCGTGCTTGA  117
               ||||||||||||||||||||||||||||||||||   ||||||||||||||||||||||
Sbjct 410       CCTTTGGGGCCCGTCCCCCCCGAAGAGGGGACGACGACCCAACACACAAGCCGTGCTTGA  349

Query 118      TGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCG  177
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 350      TGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCG  289

Query 178      TTCAA-GACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGC  236
               ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 290      TTCAAAGACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGC  229

Query 237      GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATA  296
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 230      GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATA  169

Query 297      CAATCAACTCAGACTTNACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCGGGC  356
               ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 170      CAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCGGGC  109

Query 357      CCGGGGGCTGATGCCCCCCGGCGGCCTTAAAGGCGGGCCCCGCCGAAGCAAGCAACTTAA  416
               |||||||||||||||||||||||||||||||||||| |||||||||      |||
Sbjct 110      CCGGGGGCTGATGCCCCCCGGCGGCCTTAAAGGCGGGCCC-GCCGAAGCAAC-----TAA  43

Query 417      GGTTACAGTAAACACGGGTGGGANGTTGGG  446
               ||||||||||||||||||||||| ||||||
Sbjct 44       GGTTACAGTAAACACGGGTGGGAGGTTGGG  13
```

The above rDNA ITS gene sequence data confirm that the above species is *A.tamarii*. When the same sequence in another software available online (www.mycobank.org) dedicated to fungal group only shows 50 strain of *Aspergillus,* in which shows 97.111% to 96.886% similarity it matches 437/450 bp (97.11%), Gap: 10 (2.22%), Coverage: 446/450 (99.11%), Score: 581.681, Probability: 8.40353E-167, Direction: +/-.

It also resembles with *A. bombycis* 96.653% similarity, *A. nomius* 95.923%, 94.22% with the *A. flavus* and *A. oryzae*, 93.703% similarity with *A. alliaceus* and *A. versicolor,* and the strain of *A. ochraceus* and *A. tubingenesis* shows 92.141% similarities.

When compared with Indoor dermatophytes, the software generates 50 results in which 37 strain of *A. flavus* shows 87.623% similarity. *A. bombycis, A. nomius, A. alliaceus, A. versicolor* and *A. ochraceus* shows similarity of 82.908%, 80.74% 77.8%, 55.599%, and 64.047% respectively. Final 8 strains of *A. tubingenesis* show 71.12% similarity.

When compared with *Penicillium* genus it generates 5 strains of *Penicillium allii* showing 80% similarities.

Table 1: Top five hits upon BLAST analysis

Gene bank Accession No.	Description	Max Score	Query Score	E value	Identification
KJ013529.1	*A.tamarii* strain LS 01	830	100%	0.0	98%
KJ175443.1	*A.tamarii* isolate CBS 121599	830	100%	0.0	98%
KJ175442.1	*A.tamarii* isolate CBS 118098	830	100%	0.0	98%
KJ175441.1	*A.tamarii* isolate CBS 591.68	830	100%	0.0	98%
KJ175440.1	*A.tamarii* isolate CBS 129.49	830	100%	0.0	98%

Although morphologically the *A. tamari* species resembling too much with *A. flavus*, the molecular study distinct it in a two different species. The strain of *A. tamarii* used in present study reproduces asexually by means of conidia only; perhaps some *A. tamarii* strain develops sclerotia which served for the harsh environment. The *A. tamarii* belong to class Ascomycetes but many species including *A. tamarii* unable to produce sexual spores; ascospores. Continuous hybridization leads to loss of sexual reproduction which is a problem for species identification, the suggestion for this problem was given by O' Donnell *et al.* in 2004 by considering hybridization in morphological identification. Molecular techniques make it possible to address questions about the origin and duration of asexual species in nature. ITS region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode between inter and intraspecific variation (Conrad et al. 2012).

The use of *A. tamarii* for the production of economically important metabolites and acids was carried out since long time (Dorner 1983; Tetsuhisa *et al.* 1996; Andrea *et al.* 2008) and still its importance is increasing day by day in many new emerging areas of sciences. That's why it's stability of taxonomy plays a significant practical concern. We include two types of study to reach identification, still ITS does not resolve vary closely related phylogenetic species (Bruns 2001) further genomics and proteomics studies are important for full exact identification of Individuals *Aspergillus* species.

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