Fungal keratitis is a major sight-threatening corneal infection: and mycotic keratitis is more common in tropical parts of the world including India. *Aspergillus flavus* and *Fusarium* are the predominant causative agents of corneal infection. We extracted conidial surface proteins of *A. flavus* from saprophyte and clinical isolates and analyzed the proteins using high resolution mass spectrometry. The data revealed ecotype specific alteration in surface proteome since the proteome profile of the clinical isolates and saprophyte showed significant differences. Detailed examination of the mass spec data of RodA proteins extracted from polyacrylamide gels revealed the presence of two proteoforms of this protein. We also identified the mechanism of formation of these two isoforms. Detailed analysis of this data and the conclusions derived are described in the article, “Identification of the...
proteoforms of surface localized Rod A of *A. flavus* and determination of the mechanism of proteoform generation* [1].

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1. Data

Pairwise comparison of the rodA gene of *Aspergillus flavus* and the related strain *Aspergillus oryzae* are shown in Fig. 1. Fig. 2 shows the deduced hydrophobicity plot of the RodA protein and Rod B proteins. RT-PCR data of the transcripts of rodB from mycelia grown at two different temperature is shown in Fig. 3. Spore surface proteins of saprophyte, CI1123 and CI 1678 are listed in Supplementary Tables 1–3. Supplementary Table 4 shows the pattern of conserved cysteins in RodA and Rod B proteins of *A. flavus*.

2. Experimental design, materials and methods

2.1. Preparation of samples for mass spectrometry

Previous report showed selective extraction of spore surface RodA protein of *Aspergillus fumigatus* by formic acid [2]. This procedure has been optimized as described below to avoid extraction of other surface proteins from spores. Spores were collected from CZA plates and mixed with 400 µl for 100% formic acid and incubated at 4 °C on ice for 1 h. After incubation spore suspension was centrifuged at 5000 rpm at 4 °C for 10 min, and then the supernatant was dried under a stream of nitrogen. The dried spore extract was resuspended in lysis buffer. Proteins were separated using 16% SDS PAGE and the proteins were visualized using Coomassie Brilliant Blue staining.
Fig. 1. Pairwise alignment of rodA gene of *A. flavus* and *A. oryzae* transcripts. Intron one and intron two sequences are highlighted.
2.2. Mass spectrometry of spore surface proteins

Analysis of in gel-digested proteins was done as described below. In brief, samples were electrophoresed until the dye reached 1 cm in to the separating gels. Stained gel pieces were washed twice with water and completely destained by repeated incubation in 25 mM ammonium bicarbonate prepared in 50% acetonitrile. Gels were dehydrated using 100% acetonitrile followed by reduction and alkylation [1]. After three washes in 100 µl of 100 mM ammonium bicarbonate gel pieces were dehydrated using 100% acetonitrile. Dehydrated gel pieces were dried under vacuum and were rehydrated for 30 min on ice with 600 ng of trypsin (Invitrogen) and 300 ng of glu-C (Promega) in 5 µl of 100 mM ammonium bicarbonate in 10% acetonitrile. Tryptic were then extracted from gel pieces using 25 µl of 0.1% trifluoroacetic acid (TFA) in 60% acetonitrile and then with 20 µl of 100% acetonitrile. Extracted peptides were combined and desalted using C18 tips, and stored at 4 °C. Just before analysis, the peptides were suspended in 10 µl of 0.1% FA and analyzed using Orbitrap Velos Pro mass spectrometer [3,4]. The number of surface proteins identified in each strain were comparable, with 492, 469 and 477 proteins in ATCC (Supplementary Table 1), CI 1123 (Supplementary Table 2) and CI 1698 (Supplementary Table 3), respectively. Of these, only 289 proteins were common to all three isolates.

LC-MS/MS parameters were described in detail previously [3]. All MS/MS raw data acquired from Orbitrap Velos Pro Mass Spectrometer were analyzed by Proteome Discoverer v1.4 using Mascot (Matrix Science, London, UK; version 2.4.1.0) and the inbuilt Sequest HT algorithm. Both Sequest HT and Mascot were set up to search the database containing the complete human proteome including the isoforms downloaded from the Uniprot database on 31st July 2013 (141130 entries) and its Decoy database. In addition, Raw files were also searched using PEAKS software against A. flavus database from uniport. Protein products from mRNA with introns were identified using the in vitro translated
protein sequence from the unspliced mRNA. *A. flavus* *rodA* gene sequence (NCBI gene ID 7910713) was downloaded from NCBI database and the sequence of unspliced and spliced mRNA were deduced, based on the data from closely related *A. oryzae* *rodA* sequence (Fig. 1). ExPASy Translate tool was used to convert the *rodA* mRNA sequences to amino acid sequence and used for the analysis of mass spectrometry data.

The genome of *A. flavus* encodes seven hydrophobin family proteins and their identity were confirmed by the presence of cysteine pattern and hydropathy plot (Fig. 2 and Supplementary Table 4). Data of RT PCR analysis of *rodB* mRNA was done as described in the main article [1] indicated the strain specific expression of *rodB* in cultures grown for 40 hrs at 30 °C (Fig. 3).

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**Transparency document**

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.103817.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.103817.

**References**

[1] Mohammed Razeeth Shait Mohammed, Muthu Kumar, Rabbind Singh Amrathlal, Priya Kannan, Jeya Maheshwari Jayapal, Venkatesh Prajna Namperumalsamy, Lalitha Prajna, Kuppamuthu Dharmaningam, Identification of the proteoforms of surface localized Rod A of *Aspergillus flavus* and determination of the mechanism of proteoform generation, J Proteomics 193 (2019) 62–70.

[2] S. Paris, J.P. Debeaupuis, R. Crameri, M. Carey, F. Charles, M.C. Prevost, C. Schmitt, B. Philippe, J.P. Latge, Conidial hydrophobins of *Aspergillus fumigatus*, Appl. Environ. Microbiol. 69 (2003) 1581–1588.

[3] R.M. Selvam, R. Nithya, P.N. Devi, R.S. Shree, M.V. Nila, N.L. Demonte, C. Thangavel, J.J. Maheshwari, P. Lalitha, N.V. Prajna, K. Dharmaningam, Exoproteome of *Aspergillus flavus* corneal isolates and saprophytes: identification of proteoforms of an oversecreted alkaline protease, J Proteomics 115 (2015) 23–35.

[4] J. Kandhavelu, N.L. Demonte, V.P. Namperumalsamy, L. Prajna, C. Thangavel, J.M. Jayapal, D. Kuppamuthu, *Aspergillus flavus* induced alterations in tear protein profile reveal pathogen-induced host response to fungal infection, J Proteomics 152 (2017) 13–21.