Identification Of 2-D Protein Profile Of Aspergillus Niger From Clinical Isolates

Desh Deepak Singh

Abstract - *Aspergillus niger* commonly found in air and individuals exposed by its spore, causes otomycosis infections, which can cause temporary hearing loss and leads to damage of the ear canal and tympanic membrane. Protein profile of *A. niger* were observed in 48 h cultured by using 2D-PAGE (2-Dimensional acrylamide gel electrophoresis) and MS-TOF (Mass spectroscopy -time of flight) analysis. A few 2D-PAGE protein spots were subjected to detection secondary metabolites and provides strong evidence for the analysis of multiple pathways and help in early diagnosis and treatment of the infection.

KEYWORDS: Asperigillus niger, culture, proteome, MS -TOF, 2D-PAGE, In-silico approach.

1. INTRODUCTION

Aspergillus species are the most common airborne fungi, producing allergens, causes respiratory infections such as asthma, emphysema, chronic obstructive pulmonary diseases (COPD) (Athanazio., 2012). Laboratory diagnosis of fungal infection are based on serology, cell culture and molecular methods (Kozel and Wickes,2014). In recent years, high-throughput shotgun proteomics has been used to study metabolite with the combined use of liquid chromatography and tandem mass spectrometry (LC-MS/MS) (Karpievitch et al., 2010). The aim of the present work was to the study of the intracellular proteome of *Aspergillus niger* and identified the structure of the proteins with the help of in-silico method that give insights in to the function.

2 MATERIAL AND METHODS

Aspergillus niger was obtained from MLB medical college Jhansi, India and grown on Malt Yeast 40% Glucose, agar was used throughout the study. Spores were washed with 0.1% PBS solution, counted by using Neubauer chamber and set concentration up to 1 x 10^6 spores/mL.

2.1 Inoculum Preparation

The *Aspergillus niger* conidia from 5 days culture were saturated by adding 10 ml of Diocetyl ester of sodium sulpho succinic acid to each slant. The supernatant containing conidia was transferred tainted aseptically and the interruption was used as an inoculum [5].

2.2 Fermentation Technique

The medium containing (g L^{-1}); wheat bran 5.0, $NaNO_3$, 1.0, NH_4Cl 1.5, KH_2PO_4 1.0, $MgSO_4$ $7H_2O$ and Tween 80 2.0 ml at pH 4.5 was used for fermentation in 250 ml Erlenmeyer flask. The flasks were sterilized in the autoclave at 121°C for 15 minutes (15lbs/inch^2). after 48 h mycelial protein were prepared [6].

2.3 Mycelial Preparation

Fungal mycelial was used by measuring its dry cell weight. The samples were stored at -70 °C [7].

2.4 Protein Preparation

Mycelial protein was prepared according to Oliveira et al., 2011. **[8]**

2.5Two-Dimensional Electrophoresis

2-D electrophoresis was performed according to Bhadauria and Peng,2010 [9].

2.6 Mass Spectrometry

Mass spectrometry was carried out using an MS-TOF Autoflex mass spectrometer (Brukers Daltonics, Yokohama, Japan).(http://www.matrixscience.com/search_forms_elect.htm) [10,11].

2.7 Identification of protein spots from two-dimensional gels

Protein spots were excised as described previously [11,12] Annotations are according to the sequenced genome of *A. niger* and the current NCBI Reference Sequence database (http://www.ncbi.nlm.nih.gov/refseq/, status 2020/01/06)

2.8 Mass Spectrometry

Nano Liquid chromatography-mass spectrometry (LC-MS)/MS was performed using a QSTAR-XL hybrid quadrupole-time of flight tandem mass spectrometer (Applied Biosystems), masses were then excluded from MS/MS fragmentation when the remaining aliquot of each fraction was rerun [13].

2.9 Mass Spectrometry database searching

The resulting spectra from the MS analysis of the *A. niger* samples were submitted to a local implementation of the open mass spectrometry search algorithm (OMSSA) search engine [14].

2.10FUNCTIONAL ANNOTATION GROUPS AND SEQUENCE ANALYSIS

Protein similarity analyses were done using the Clustal W and Jalview version 2 tools Trans membrane domains (TMD) were assessed using the TMHMM tool [15].

- *Dr. Desh Deepak Singh, Amity institute of Biotechnology, Amity University Rajasthan , SP-1, Kant Kalwar, RIICO Industrial Area, NH-11C, Jaipur, Rajasthan, India. Email:ddsbms@gmail.com, dds_bms2002@rediffmail.com*

2.11Relative protein abundance and statistics.
A normalized spectral abundance factor was used as a parameter to estimate relative protein abundance [16].

2.12 Protein sequence retrieval and analysis:
Target proteins were retrieved from National Centre for Biotechnology Information database (NCBI) and aligned using Clustal W (http://www.genome.jp/tools-bin/clustalw) software to determine the appropriate sequence for protein structure prediction (http://www.genome.jp/tools-bin/clustalw) [16,17]

2.13 Insilico modelling:
The similarity of the proteins included in our study was compared with the available protein homologs against non-redundant databases like BLASTP program of NCBI and PDB (https://swissmodel.expasy.org/) [18].

2.14 Structure validation and refinement:
The quality of the structure was determined using QMEAN6 program of the SWISS-MODEL workspace. Finally, the modeled structures were visualized using PyMOL v1.7.4.5 [19].

3. RESULT AND DISCUSSION
2-D protein profile of A. *niger* are shown in figure 1, Spots 2,10, 25, and 26 were observed as hypothetic protein. Spot 3 and 9 are mitochondrial protein, spot 4 is related to cytochrome C of A. *niger*. Spot 10 matched to A. *niger* reductase. Spot 25 identified as putative β-xylosidase of A. *niger*. Spot 26 is related to AMP deaminases of A. *niger*. Spot 16 matches putative calcium P-type ATPase to A. *niger*, while protein 21 matches as α-sarcin. Protein identification through MS patterns generally depends on the quality of the annotated gene sequences available in the database banks [19,20,21]. Various Protein spot were identified in 2D Gel as described below-
Spot no: 3: Mr(Da): *28891****; pI:*** *9;* ***accession*** *no.: gi | 40741129;* ***NCBI Protein description****: Hypothetical protein AN4402.2 [Aspergillus nidulans FGSC A4]. 284 aa.;* ***Query coverage:*** *100%;* ***Related proteins:*** *Outer mitochondrial membrane protein porin;* ***E value*** *[Neosartorya fischeri NRRL 181] (2e-48);* ***Searched/ matched peptides:*** *19/8;* ***Sequence Coverage:*** *34%.*
Spot no: 4: Mr(Da): *47605****; pI: 9****;* ***accession*** *no.: gi | 40739821;* ***NCBI Protein description****: Hypothetical protein AN8273.2[Aspergillus nidulans FGSC A4].;* ***Query coverage:*** *89%;* ***Related proteins*** *Ubiquinol-cytochrome C reductase complex core protein 2, putative;* ***E value*** *[Aspergillus fumigatus Af293] (1e-138);* ***Searched/ matched peptides:*** *18/5;* ***Sequence Coverage:*** *28%.*
Spot no: 10: Mr(Da): *51220****; pI:*** *8.79;* ***accession*** *no.: gi|40739937;* ***NCBI Protein description****: Hypothetical protein AN3862.2[Aspergillus nidulans FGSC A4]. 468 aa..;* ***Query coverage:*** *49%;* ***Related proteins:*** *Cytochrome b5 reductase, putative ;* ***E value*** *[Aspergillus flavus NRRL3357] (6e-174);* ***Searched/ matched peptides:*** *9/4;* ***Sequence Coverage:*** *44%.*
Spot no: 12: Mr(Da): *50549****; pI: 8.****93;* ***accession*** *no.: gi | 40738989;* ***NCBI Protein description****: Hypothetical protein AN6650.2 [Aspergillus nidulans FGSC A4]. 460 aa.;* ***Query coverage:*** *94%;* ***Related proteins*** *Hypothetical protein;* ***E value*** *Hypothetical protein [Aspergillus oryzae RIB40] (2e-159);* ***Searched/ matched peptides:*** *10/4;* ***Sequence Coverage:*** *51%.*
Spot no: 13: Mr(Da): 43391**; pI: 6.**91; **accession** no.: gi | 40743549; **NCBI Protein description**: Hypothetical protein AN5646.2 [*Aspergillus nidulans* FGSC A4]. 417 aa.; **Query coverage:** 92%; **Related proteins** Hypothetical protein An04g05720; **E value** [*Aspergillus niger*] (9e-137) ; **Searched/ matched peptides: 1**9/7; **Sequence Coverage:** 55%.
Spot no: 17 Mr(Da): 60416 **; pI:** 8.13 gi | **accession** no.: 40745893; **NCBI Protein description** Hypothetical protein AN1884.2 [*Aspergillus nidulans* FGSC A4].544 aa. **Query coverage:** 81%; **Related proteins:** Cytochrome P450 monooxygenase, putative; **E value**[*Aspergillus fumigatus* A1163](4e-33); **Searched/ matched peptides:** 12/5; **Sequence Coverage:** 25%.
Spot no: 25 Mr(Da): 12378**; pI:** 7.85gi | **accession** no.: 40743794;**NCBIProtein description**: FGSC A4]. 110 aa. Hypothetical protein AN2633.2[*Aspergillus nidulans;* **Query coverage:** 62%; **Related proteins:** Hypothetical protein AN7864.2; **E value** [*Aspergillus nidulans* FGSC A4] (3e-26); **Searched/ matched peptides:** 8/3; **Sequence Coverage:** 45%.
Spot no: 20: Mr(Da): 78048**; pI: 8.**83; **accession** no.: gi | 40739380; **NCBI Protein description**: Hypothetical protein AN6752.2 [*Aspergillus nidulans* FGSC A4].696 aa.; **Query coverage:** 94%; **Related proteins** putative **E value** [*Aspergillus fumigatus* A1163] (1e-138); S**earched/ matched peptides: 1**4/6; **Sequence Coverage:** 48%.
Spot no: 25: Mr(Da): 12378**; pI: 7.85**; **accession** no.: gi | 40743794; **NCBI Protein description**: Hypothetical protein AN2633.2 [*Aspergillus nidulans* FGSC A4]. 110 aa.; **Query coverage:** 62%; **Related proteins** Hypothetical protein AN7864.2; **E value** [*Aspergillus nidulans* FGSC A4] (3e-26); S**earched/ matched peptides: 8**/3; **Sequence Coverage:** 45%. The above identified protein may help in identification of host pathogen interaction (Fig. 1).

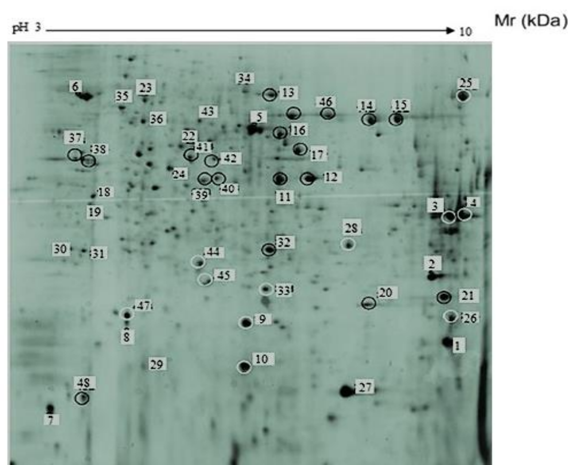

Fig. 1 Silver-stained 2-DE gels of intracellular *Aspergillus niger* biofilm proteins.

4. CONCLUSIONS

A. niger has revealed expression of multiple proteins and provides strong evidence for the analysis of multiple pathways. These proteins responsible for morphogenetic and physiological responses derived from this biological process. The results of the various protein spot are tools used in the analysis of 3D modeling of proteins indicate acceptable model quality and similar structures may exist in nature. The results of this study can impact the *A. niger* development, antifungal drug discovery, diagnostic biomarker assessment as well as Aspergillus *niger* pathogenesis. These proteins may be used for the identification of active infection, as well as possible new therapeutic targets.

ACKNOWLEDGEMENTS:

The author gratefully acknowledges Proteomics and Structural Genomics lab, Animal Biotechnology Division, NDRI, Karnal India; Institute of Genomics Integrative Biology, Mall Road Delhi University, Camus Delhi India, Department of Microbiology King George's Medical University, Lucknow, India; Dr. Rambir Singh, Institute of Biomedical sciences, Bundelkhand University Jhansi India; Department of Chemistry, Banaras Hindu university, Varanasi India, Amity Institute of Biotechnology, Amity University Rajasthan, India for their wonderful lab support without any of them this work was not possible for me.

REFERENCES

[1] C. Paulussen, J.E. Hallsworth, S. Álvarez-Pérez, et al. Ecology of aspergillosis: insights into the pathogenic potency of Aspergillus fumigatus and some other Aspergillus species. Microbiology Biotechnology, Vol.2, pp. 296–322,2017

[2] Kozel, R. Thomas, Brian Wickes. Fungal diagnostics. *Cold Spring Harbor* Perspectives in Medicine. vol. 4, pp 4, 2014

[3] Karpievitch, V. Yuliya et al. "Liquid Chromatography Mass Spectrometry-Based Proteomics: Biological and Technological Aspects." The annals of applied statistics vol. 4, 1797-1823, 2010

[4] Novodvorska, Michaela et al. "Metabolic activity in dormant conidia of Aspergillus niger and developmental changes during conidial outgrowth." Fungal genetics and biology: FG & B vol. 94, PP 23-31, 2016

[5] K. Hayer, M. Stratford, D.B. Archer. Germination of Aspergillus niger conidia is triggered by nitrogen compounds related to L-amino acids. Appl Environmental Microbiology Vol 19, pp 6046–6053, 2014

[6] Sonawane, Kailas D et al. Intergeneric fusant development using chitinase preparation of Rhizopus stolonifer NCIM 880." AMB Express vol. 6, pp 114, 2016.

[7] H. Du, P. Lv, M. Ayouz, A. Besserer, P.Perré . Morphological Characterization and Quantification of the Mycelial Growth of the Brown-Rot Fungus Postia placenta for Modeling Purposes published correction appears in PLoS One. Vol, 5, pp.30,2018

[8] De. Oliveira, P. José, M. Ferreira, H. Leo, de Graaff. "Proteomics of industrial fungi: trends and insights for biotechnology." Applied microbiology and biotechnology vol. 2,pp 225-37, 2011.

[9] Vödisch,, Martin et al. "Analysis of the Aspergillus fumigatus proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia." Journal of proteome research vol.5, pp 2508-245, 2011.

[10] Prentice, M Boone et al. "High-speed MALDI MS/MS imaging mass spectrometry using continuous raster sampling." Journal of mass spectrometry JMS, vol.4, pp 703-10, 2015.

[11] A. Yoshinaga, H. Kamitakahara, K. Takabe Distribution of coniferin in differentiating normal and compression woods using MALDI mass spectrometric imaging coupled with osmium tetroxide vapor treatment. Tree Physiology 36 (5) 643–6522016.

[12] Rodrigues, P Elisete, et al. "A simple, economical and reproducible protein extraction protocol for proteomics studies of soybean roots." Genetics and molecular biology vol.1, pp 348-52.

[13] Pitt, J. James "Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry." The Clinical biochemistry. Reviews vol. 1, pp 19-34, 2009.

[14] Yang, Yong et al. A comparison of nLC-ESI-MS/MS and nLC-MALDI-MS/MS for GeLC-based protein identification and iTRAQ-based shotgun quantitative proteomics." Journal of biomolecular techniques*:* JBT vol. 18, pp 4, 2007.

[15] Punta, Marco et al. Membrane protein prediction methods." Methods (San Diego, Calif.) vol. 4, pp 460-74, 2007.

[16] McIlwain, Sean et al. "Estimating relative abundances of proteins from shotgun proteomics data." BMC bioinformatics vol. 13,pp308, 2012.

[17] E W Sayers, T. Barrett, D. A. Benson, E. Bolton, S. H. Bryan, Canes K, Ye J. 2011. Database resources of the National Center for Biotechnology Information. *Nucleic acids research*, *39*(Database issue), D38–D51.

[18] Manochitra, Kumar, Subhash Chandra Parija. In-silico prediction and modeling of the Entamoeba histolytica proteins: Serine-rich Entamoeba histolytica protein and 29 kDa Cysteine-rich protease. *PeerJ* vol.5,pp 28, 2017

[19] Bordoli, Lorenza, T. Schwede. Automated protein structure modeling with SWISS-MODEL Workspace and the Protein Model Portal. Methods in molecular biology vol. 857, pp 107-36, 2012

[20] Alford SC, Pearson JD, Carette A, Ingham RJ, Howard PL. Alpha-sarcin catalytic activity is not required for cytotoxicity. *BMC Biochem*. 2009;10:9. Published 2009 Apr 3. doi:10.1186/1471-2091-10-9