Identification of protein drug targets for Aspergillosis caused by A. niger using in-silico method - SPA approach

Sofia Khatoon[a], Suchitra Ku Panigrahy[a], Sushma Dubey[a*]

[a]Department of Biotechnology, Kalinga University, Naya Raipur, Chhattisgarh-492101, India

[*]Email: sushma.dubey@kalingauniversity.ac.in

Abstract

A. niger is caused invasive fungal infections have been associated with serious health issues. Aspergillosis resistance is brought on by the combination of triazoles (Voriconazole, Posaconazole, and Intraconazole) with antifungals (Micafungin, Caspofungin, Amp B, etc.); new antifungal medications are therefore required for the treatment of this condition. An *in-silico* computational method for retrieving new targets is the SPA approach. Several bioinformatics servers and programmes have been used to perform Subtractive Proteome Analysis on the full proteome of *A. niger* (strain CBS 513.88 / FGSC A1513). From UNIPROT, the total number of proteins was gathered, and redundant sequences were eliminated using the CD-HIT method. Following the use of the Basic Local Alignment Search Tool (BLAST) and a collection of databases for important genes (DEG), the proteins that were non-homologous to humans and bacteria were discovered for metabolic pathway analysis (KEGG). Calculating the number of target proteins is possible following the retrieval of unique identification routes. DrugBank was used to aid in the druggability analysis. The BUSCA and PSORT II web portals were used to find the proteins' locations. The new Aspergillosis targets were correctly predicted.

Keywords

Aspergillosis, triazoles SPA approach *A. niger*

Introduction

A fungus called *Aspergillus* species is the root cause of aspergillosis. A eukaryotic organism, Aspergillus. Only a small number of the known more than 180 species can cause aspergillosis, which can occasionally be fatal, especially in people with weakened immune systems, those who have had surgery, or those who have undergone organ transplantation. Aspergillosis was treated with a combination of triazoles like Voriconazole, Posaconazole, and Itraconazole and antifungal medications like Caspofungin, Micafungin, and Amphotericin B. Later, *Aspergillus* species developed resistance to azole combinations and antifungal medications. New prospective medications were therefore required. For the purpose of identifying new protein therapeutic targets for the treatment of particular diseases, the Subtractive Proteome Analysis (SPA) approach, an *in-silico* computer technique, was introduced. The SPA technique was used for the species *A. niger* since triazoles and antifungal medications were no longer effective against

Aspergillus species (superbug). The SPA method produced a list of unique drug targets that can be employed in the pharmaceutical business and for future study.

Materials and Methods

Proteome Retrieval

The initial stage of the Subtractive Proteome Analysis (SPA) method is to gather all of the species protein sequences. The following computational web portal and databases were used for this: UniProt (Universal Protein Resources). FASTA files for *A. niger* (strain CBS 513.88/FGSC A1513) were stored.

Removal of Redundant or Paralogous Sequences

A large number of useless or non-essential proteins were recorded in FASTA format with the proteome data. As a result, the duplicated sequences for the selected strain were removed using the bioinformatics tool CD-HIT. At 0.6 (60) cut-off, the sequence identity parameter was set. The duplicated sequences were eliminated following CD-HIT. There were just necessary sequences picked.

Retrieval of Essential Proteins

Collecting necessary proteins is the third phase. To break down living tissues, fungi use enzymes, which results in disease. The Database of Essential Genes (DEG) (version 15.2) was used to retrieve the necessary proteins. The protein database uses NCBI (National Centre for Biotechnology Information) to transform the extracted essential protein sequences into FASTA format.

Non – Homologous Protein Identification

BLAST and the National Library of Medicine (NCBI) are both included on an official US government website. This programme compares the similarities between nucleotide or protein sequences and provides the necessary information. The FASTA-formatted essential proteins retrieved by DEG are passed to the BLASTp suite. Non-redundant protein sequences (nr) were the target of the conditions applied to databases after standard databases (nr, etc.). Aspergillus (taxid:5052) was selected as the organism, and the protein-protein BLAST method, BLASTp, was used. The BLAST was finally executed.

Metabolic Pathway Identification

The Genome Database or Kyoto Encyclopedia of Genes and Genomes (KEGG) database provides details on the particular metabolic route of the species and Homo sapiens. Only those pathways that were present in the Aspergillus species and not in Homo sapiens were chosen from the BLASTp suite result that was associated to the KEGG database. The prospective protein drug targets reached this point in their development.

Subcellular Localization Prediction

Protein localization was predicted using PSORT II. Because it verifies where the proteins are, which is also necessary for the targets, localization is significant.

Druggability Scrutiny

The collected KEGG data of novel proteins was examined, specifically their druggability potential, or if the proteins targeted are druggable or not and FDA approved. This analysis was performed using the DrugBank computational tool.

SUBSTRACTIVE PROTEOME ANALYSIS FOR IDENTIFICATION OF DRUG TARGET		
STEPS	**TOOLS USED**	**REFRENCES**
Step 1 – Proteome Retrieval	**NCBI, UniProt**	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277342/
Step 2 – Removal of Paralogous or Redundant Sequences	**CD-HIT**	http://www.eurekaselect.com/article/102083
Step 3 – Retrieval of Essential Protein	**Geptop 2.0 server, DEG**	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277342/
Step 4 – Essential Non – Homologous Protein Identification	**BLASTp**	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277342/
Step 5 – Unique Pathway Identification	**KEGG, Genome Database**	https://www.researchgate.net/publication/337134834_Subtractive_Proteome_Analysis_of_Candida_albicans_Divulges_Promising_Antifungal_Targets
Step 6 – Subcellular Localization Prediction	**BUSCA, PSORT II, CELLO**	http://www.eurekaselect.com/article/102083
Step 7 – Druggability Analysis	**DrugBank**	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277342/

Fig 1. Systematic arrangement of Subtractive Protein Analysis approach steps (7) with bioinformatics tool used and links.

Results and Discussion

The goal of the entire investigation was to identify brand-new pharmacological targets responsible for human aspergillosis. *A. niger* (strain CBS 513.88 / FGSC A1513) was given the SPA treatment.

PROTEIN RECOUPMENT & DECIMATION OF REDUNDANT SEQUENCES

We obtained the complete proteome from UniProt. The strain of *A. niger* (strain CBS 513.88 / FGSC A1513) had 124053 total proteins. The proteins from the UniProt results were stored in FASTA format.

Using the computational tool CD-HIT suite, these 124053 were reduced to 123817 proteins. Sequence identity parameter, 60% (0.6) cut-off, was the filter used to run CD-HIT. Additionally, the CD-HIT suite's output in FASTA format was downloaded.

RECOVERY OF ESSENTIAL GENES & NON-HOMOLOGOUS MATCHES

The essential genes were gathered from DEG (Database of Essential Genes) version 15.2. The gene present must be able to survive in the pathogenic host. This aids in directing the new medications. Using NCBI's Protein Database filter, the list of important genes was transformed into FASTA format. 161 crucial genes were found.

For non-homologous matches, BLASTp suite (Protein-Protein BLAST) was used. The 86 important protein sequences can be compared to one another using BLASTp, which also calculates the total number of non-homologous sequences. Standard database (nr, etc.), non-redundant protein sequences (nr), organism (optional) Aspergillus (taxid:5052), and programme selection at BLASTp (protein-protein BLAST) were the parameters before starting the BLASTp suite process. 2204 sequences were found in the non-homologous BLASTp results.

Table: 1 Catalogue of Essential genes of A. niger

S.No.	*A. niger*
1.	Hypothetical protein KXX42 005253 [Aspergillus fumigatus]
2.	Extracellular polygalacturonase, putative [Aspergillus fumigatus]
3.	Extracellular endo-polygalacturonase, putative [Aspergillus fumigatus]
4.	Extracellular exo-polygalacturonase, putative [Aspergillus fumigatus Af293]
5.	DEAD-box ATP-dependent RNA helicase [Aspergillus fumigatus]
6.	Pre-mRNA processing RNA – helicase [Aspergillus fumigatus]
7.	RNA-dependent ATPase [Aspergillus fumigatus]
8.	Ribosomal RNA processing protein [Aspergillus fumigatus]

9.	Eukaryotic translation initiation factor Eif-4A subunit, putative [Aspergillus fumigatus]
10.	ATP dependent helicase (Dbp10), putative [Aspergillus fumigatus]
11.	Exportin-1 [Aspergillus fumigatus]
12.	Putative type 1 phosphatase regulator ypi1 [Aspergillus fumigatus]
13.	Vesicle coat component [Aspergillus fumigatus]
14.	Conserved hypothetical protein [Aspergillus fumigatus]
15.	COPII vesicle coat protein Sec16,, putative [Aspergillus fumigatus]
16.	C6 transcription factor (Ctf1B), putative [Aspergillus fumigatus]
17.	ABC multidrug transporter, putative [Aspergillus fumigatus A1163]
18.	ABC drug exporter AbcA [Aspergillus fumigatus Af293]
19.	Serine/threoninee protein kinase Kin4 [Aspergillus fumigatus var. RP-2014]
20.	NACHT and Ankyrin domain protein [Aspergillus fumigatus Af293]
21.	WSC domain-containing protein, putative [Aspergillus fumigatus Z5]
22.	Metal homeostatis protein bsd2 [Aspergillus fumigatus var. RP-2014]
23.	Fungal specific transcription factor, putative [Aspergillus fumigatus Af293]
24.	Chromatin structure remodeling complex protein RSC3, putative [Aspergillus fumigatus A1163]
25.	Transcriptional regulator, putative [Aspergillus fumigatus]
26.	Cytochrome P450 sterol C-22 desaturase, putaitve [Aspergillus fumigatus Af293]
27.	Sterol 14-alpha demethylase [Aspergillus fumigatus]
28.	Cyp51A [Aspergillus fumigatus]
29.	Zinc knuckle transcription factor/splicing factor MSL5/ZFM1, putative [Aspergillus fumigatus Af293]
30.	C2H2 transcription factor (Sfp1), putative [Aspergillus fumigatus Af293]
31.	GATA-type sexual development transcription factor Nsdd [Aspergillus fumigatus Af293]
32.	Siderophore transcription factor SreA [Aspergillus fumigatus Af293]
33.	Heat shock protein Hsp20/Hsp26, putative [Aspergillus fumigatus Af293]
34.	Endo-1,3-beta-glucanase Engl1 [Aspergillus fumigatus Af293]
35.	NEDD8 activating enzyme (UbaC), putative [Aspergillus fumigatus Af293]

36.	Ubiquitin-like activating enzyme (UbaB), putative [Aspergillus fumigatus Af293]
37.	E1 ubiquitin-activating protein [Aspergillus fumigatus]
38.	SPS-sensor component ptr3 [Aspergillus fumigatus]
39.	PolyA+ RNA transport protein UbaA [Aspergillus fumigatus var. RP-2014]
40.	Urmylation protein [Aspergillus fumigatus]
41.	Molybdenum cofactor biosynthetic protein (CnxF), putative [Aspergillus fumigatus Af293]
42.	AP-3 adaptor complex subunit beta, putative [Aspergillus fumigatus Af293]
43.	AP-1 complex subunit beta-1 [Aspergillus fumigatus]
44.	AP-2 adaptor complex subunit beta, putative [Aspergillus fumigatus Af293]
45.	Aromatic amino acid aminotransferase, putative [Aspergillus fumigatus A1163]
46.	Nonribosomal peptide synthetase 1 [Aspergillus fumigatus]
47.	MFS multidrug transporter [Aspergillus fumigatus var. RP-2014]
48.	Protein phosphatase [Aspergillus fumigatus Af293]
49.	SET and WW domain protein [Aspergillus fumigatus A1163]
50.	ZIP metal ion transporter, putative [Aspergillus fumigatus Af293]
51.	Cytokinesis protein SepA/Bni1 [Aspergillus fumigatus A1163]
52.	Diphthine synthase, putative [Aspergillus fumigatus Af293]
53.	Malate dehydrogenase, NAD-dependent [Aspergillus fumigatus Af293]
54.	Multidrug-resistance transporte [Aspergillus fumigatus]
55.	GTPase-activating protein [Aspergillus fumigatus]
56.	Vacuolar ABC heavy metal transporter (Hmt1), putative [Aspergillus fumigatus A1163]
57.	Iron-sulfur clusters transporter atm1, mitochondrial [Aspergillus fumigatus]
58.	Regulator of gluconeogenesis Rmd5, putative [Aspergillus fumigatus Af293]
59.	GID complex subunit containing RING finger motif [Aspergillus fumigatus]
60.	Citrinin biosynthesis cluster MFS transporter mrr1 [Aspergillus fumigatus]
61.	Alpha mannosidase 1A [Aspergillus fumigatus var. RP-2014]
62.	CAIB/BAIF family enzyme [Aspergillus fumigatus A1163]
63.	Heat shock transcription factor Hsf1, putative [Aspergillus fumigatus A1163]
64.	Stress response regulator/HFS transcription factor, putative [Aspergillus fumigatus

	A1163]
65.	Transposase [Aspergillus fumigatus]
66.	High-affinity hexose transporter, putative [Aspergillus fumigatus Af293]
67.	MFS sugar transporter, putative [Aspergillus fumigatus A1163]
68.	HLH transcription factor, putative [Aspergillus fumigatus A1163]
69.	SrbA [Aspergillus fumigatus]
70.	HLH DNA binding domain protein, putative [Aspergillus fumigatus Af293]
71.	Decapping enzyme Dcp2, putative [Aspergillus fumigatus A1163]
72.	Acetyltransferase, GNAT family, putative [Aspergillus fumigatus Af293]
73.	Streptothricin acetyltransferase [Aspergillus fumigatus]
74.	U3 small nucleolar ribonucleoprotein protein Mpp10 [Aspergillus fumigatus Af293]
75.	Cation transporting ATPase, putative [Aspergillus fumigatus Af293]
76.	P-type ATPase, putative [Aspergillus fumigatus Af293]
77.	ATPase P [Aspergillus fumigatus var. RP-2014]
78.	PMR1 calcium ATPase [Aspergillus fumigatus]
79.	Calcium/mangenease P-type ATPase, putative [Aspergillus fumigatus Af293]
80.	Sodium P-type ATPase, putative [Aspergillus fumigatus Af293]
81.	Putative potassium transport system protein kup 2 [Aspergillus fumigatus]
82.	Endosome to Golgi transport protein [Aspergillus fumigatus]
83.	Cellular morphogenesis regulator DopA [Aspergillus fumigatus A1163]
84.	Importin beta 4 subunit [Aspergillus fumigatus var. RP-2014]
85.	Dienelactone hydrolase [Aspergillus fumigatus Af293]
86.	Ran exchange factor Prp20/Pim1, putative [Aspergillus fumigatus Af293]
87.	Riboflavin-specific deaminase [Aspergillus fumigatus Z5]
88.	Mitochondrial protein Fmp25, putative [Aspergillus fumigatus A1163]
89.	BTB domain and ankyrin repeat protein [Aspergillus fumigatus Af293]
90.	NmrA family transcriptional regulator, putative [Aspergillus fumigatus Af293]
91.	NmrA-like family protein [Aspergillus fumigatus A1163]
92.	Nucleoside diphosphate sugar epimerase [Aspergillus fumigatus var. RP-2014]
93.	Nitrogen metabolite repression regulator NmrA [Aspergillus fumigatus Af293]
94.	Histidinol dehydrogenase, putative [Aspergillus fumigatus A1163]

95.	Imidazoleglycerol-phosphate dehydratase [Aspergillus fumigatus]
96.	Phosphoribosyl-AMP cyclohydrolase, putative [Aspergillus fumigatus Af293]
97.	DNA-directed RNA polymerase III subunit [Aspergillus fumigatus]
98.	DNA-dependent RNA polymerase II [Aspergillus fumigatus]
99.	RNA polymerase beta [Aspergillus fumigatus]
100.	RNA polymerase second largest subunit [Aspergillus fumigatus]
101.	Pre-rRNA processing protein Mrd1, putative [Aspergillus fumigatus A1163]
102.	Multiple RNA-binding domain-containing protein 1 [Aspergillus fumigatus]
103.	Protein phosphatase PP2A regulatory subunit B [Aspergillus fumigatus]
104.	Glycine-rich RNA-binding protein, putative [Aspergillus fumigatus Af293]
105.	RNA splicing factor (Pad-1), putative [Aspergillus fumigatus Af293]
106.	Eukaryotic translation initiation factor 3 subunit EifCg, putative [Aspergillus fumigatus Af293]
107.	Translation initiation factor eIF3 subunit g [Aspergillus fumigatus]
108.	RNA binding protein (Rbm8A), putative [Aspergillus fumigatus Af293]
109.	Nuclear cap-binding protein subunit 2 [Aspergillus fumigatus]
110.	Peptidyl prolyl cis-trans isomerase Cyclophilin, putative [Aspergillus fumigatus Af293]
111.	Ribonucleoprotein chloroplast [Aspergillus fumigatus var. RP-2014]
112.	Chromosome segregation protein Spc105, putative [Aspergillus fumigatus Af293]
113.	RING finger domain protein, putative [Aspergillus fumigatus A1163]
114.	Peroxisome biosynthesis protein (Peroxin-10), putative [Aspergillus fumigatus A1163]
115.	SH3 domain protein, putative [Aspergillus fumigatus Af293]
116.	Hsp70 chaperone Hsp88 [Aspergillus fumigatus Af293]
117.	Molecular chaperone Hsp70 [Aspergillus fumigatus Af293]
118.	Neutral/alkaline nonlysosomal ceramidase, putative [Aspergillus fumigatus Af293]
119.	Pyruvate carboxylase, putative [Aspergillus fumigatus Af293]
120.	SNF2 family helicase/ATPase, putative [Aspergillus fumigatus Af293]
121.	DNA helicase rad5 [Aspergillus fumigatus]
122.	DNA excision repair protein (Rad5), putative [Aspergillus fumigatus Af293]

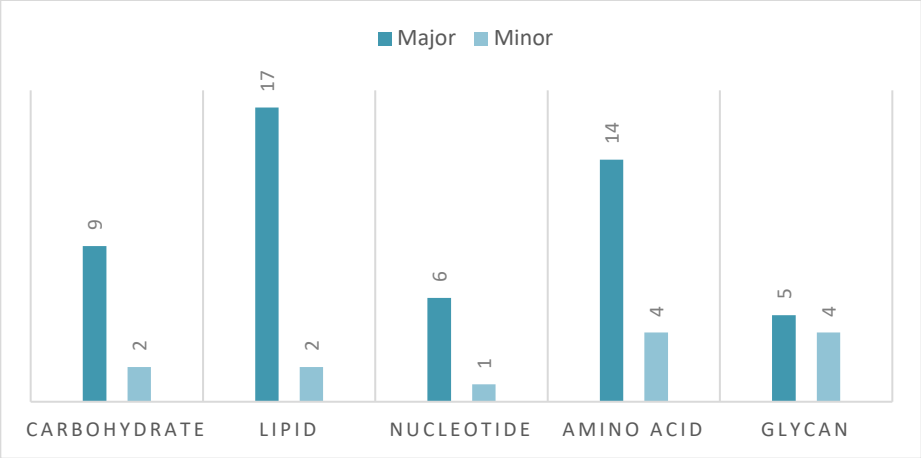
123.	SWI/SNF family DNA-dependent ATPase Ris1, putative [Aspergillus fumigatus A1163]
124.	DNA N-glycosylase, putative [Aspergillus fumigatus Af293]
125.	Anthranilate phosphoribosyltransferase, putative [Aspergillus fumigatus A1163]
126.	Non-reducing polyketide synthase pyr2 [Aspergillus fumigatus]
127.	LovB-like polyketide synthase, putative [Aspergillus fumigatus Af293]
128.	Hybrid PKS/NRPS enzyme EqiS-like, putative [Aspergillus fumigatus A1163]
129.	Pseurotin A precursor [Aspergillus fumigatus Af293]
130.	TBC domain-containing putative [Aspergillus fumigatus Z5]
131.	GTPase activating protein (Gyp1), putative [Aspergillus fumigatus A1163]
132.	Pantothenate transporter [Aspergillus fumigatus Z5]
133.	MFS transporter Liz1/Seo1, putative [Aspergillus fumigatus Af293]
134.	Phospholipid-translocating P-type ATPase domain-containing protein [Aspergillus fumigatus Af293]
135.	Haloacid dehalogenase hydrolase [Aspergillus fumigatus var. RP-2014]
136.	ATPase H /K alpha subunit [Aspergillus fumigatus var. RP-2014]
137.	AflR-like C6 transcription factor, putative [Aspergillus fumigatus Af293]
138.	Golgi matrix protein, putative [Aspergillus fumigatus Af293]
139.	TOR pathway phosphatidylinositol 3-kinase TorA, putative [Aspergillus fumigatus Af293]
140.	Inositol kinase (UvsB), putative [Aspergillus fumigatus Af293]
141.	NACHT and TPR domain protein [Aspergillus fumigatus A1163]
142.	MFS nicotinic acid transporter Tna1, putative [Aspergillus fumigatus Af293]
143.	Transmembrane transporter, putative [Aspergillus fumigatus A1163]
144.	AflT-like major facilitator superfamily protein, putative [Aspergillus fumigatus]
145.	MFS toxin efflux pump, putative [Aspergillus fumigatus Z5]
146.	MFS gliotoxin efflux transporter GliA [Aspergillus fumigatus Af293]
147.	MFS aflatoxin efflux pump, putative [Aspergillus fumigatus A1163]
148.	Cysteine-binding protein FliY [Aspergillus fumigatus Z5]
149.	TPR repeat protein [Aspergillus fumigatus Z5]
150.	TPR domain-containing protein [Aspergillus fumigatus Z5]

151.	LipA and NB-ARC domain protein [Aspergillus fumigatus Af293]
152.	nuclear export protein Noc3 [Aspergillus fumigatus Af293]
153.	Class V chitinase, putative [Aspergillus fumigatus A1163]
154.	Chi100 [Aspergillus fumigatus]
155.	MAP kinase (Mkk2), putative [Aspergillus fumigatus Af293]
156.	Ste20-like serine/threonine protein kinase, putative [Aspergillus fumigatus Af293]
157.	Mst3-like protein kinase, putative [Aspergillus fumigatus A1163]
158.	Signal transducing kinase of the PAK [Aspergillus fumigatus]
159.	Protein kinase (Chm1), putative [Aspergillus fumigatus Af293]
160.	Hexokinase [Aspergillus fumigatus Z5]
161.	GlucokinaseGlkA, putative [Aspergillus fumigatus Af293]

KEGG (KYOTO ENCLYCLOPEDIA OF GENE AND GENOEME) PATHWAY IDENTIFICATION

The crucial and final element of the SPA technique is pathway analysis. It shows the species' possible pharmacological targets. It outlines the particular metabolic route that Aspergillus species and Homo sapiens use. The proteins that were only found in Aspergillus species were chosen, but not in Homo sapiens. Since they make up the therapeutic targets, only proteins from Aspergillus species are counted. Out of 49 major and 9 minor proteins chosen as possible therapeutic targets, a total of 17 pathways were identified. Protein distribution in the pathway was only found in the areas of carbohydrate, lipid, nucleotide, amino acid, and glycan metabolism.

Table: 2 Major and Minor pathways collected from KEGG website



SUBCELLULAR LOCALIZATION PREDICTION

BUSCA or PSORT II decides on the localization identification. The PSORT II's search panel receives the KEGG pathway results in FASTA format. The PSORT II predicts the location of eukaryotic sequences. The breakdown is 65.2% of plasma membrane, 8.7% of vascular membrane, 8.7% of nuclear membrane, 4.3% of Golgi membrane, 8.7% of cytoplasmic, and 4.3% of endoplasmic reticulum.

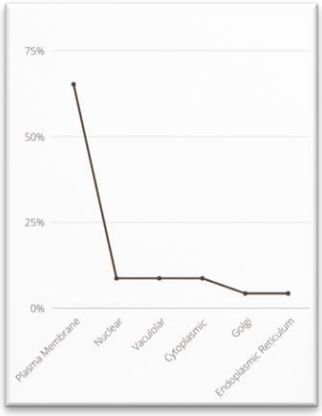


Fig 2. Localization arrangement of proteins extracted from KEGG Pathway Database. 78.3% - plasma membrane, 8.7% - vascular membrane, 4.3% - nuclear membrane, 4.3% - Golgi membrane and 4.3% - endoplasmic reticulum.

DRUGGABILITY OF POTENTIAL TARGETS

DrugBank discovered an *A. niger* druggability analysis. Nine *A. niger* proteins were found as a consequence. Information on medications, drug targets, drug interactions, pharmacology, chemical structures, metabolism, and more can be found in the freely accessible online database known as DrugBank.

1.	Hypothetical protein KXX42 005253 [Aspergillus fumigatus]
2.	Cyp51A [Aspergillus fumigatus]
3.	Siderophore transcription factor SreA [Aspergillus fumigatus Af293]
4.	Multidrug-resistance transporte [Aspergillus fumigatus]
5.	Riboflavin-specific deaminase [Aspergillus fumigatus Z5]
6.	Chi100 [Aspergillus fumigatus]
7.	Hsp70 chaperone Hsp88 [Aspergillus fumigatus Af293]
8.	Imidazoleglycerol-phosphate dehydratase [Aspergillus fumigatus]
9.	NEDD8 activating enzyme (UbaC), putative [Aspergillus fumigatus Af293]

Fig. 3 List of Potential Drug Targets (9)

Conclusion

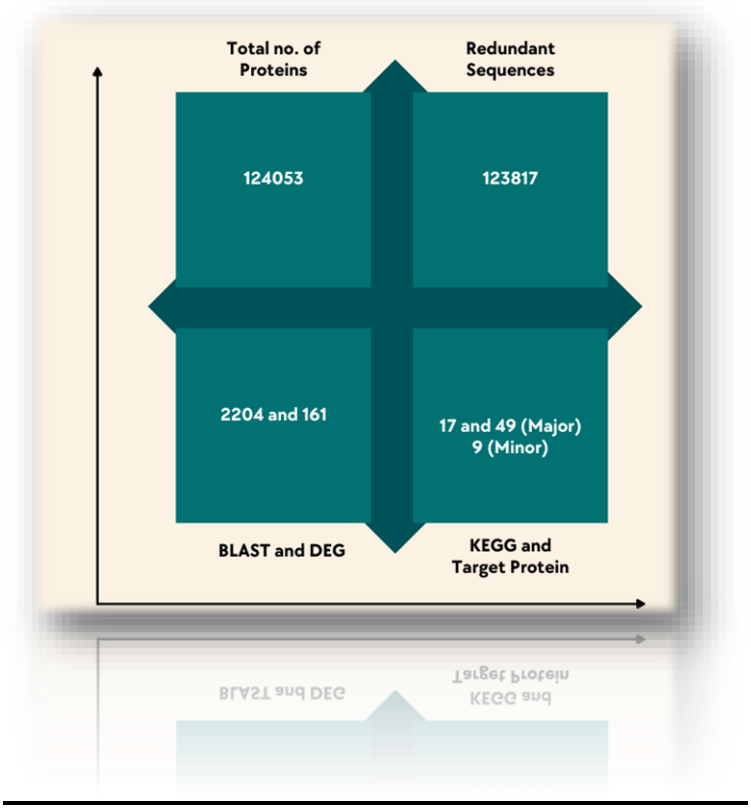


Fig 4. Bioinfographics – *A. niger*

Aspergillosis has been brought on by Aspergillus infections. By producing several invasive diseases, especially in immunocompromised people, the airborne fungus *A. niger* poses a significant health risk to humans. Because of azole and antifungal resistance (superbug), the currently used treatment for Aspergillus has become ineffective. Thus, both potential medications and fresh targets are required. This led to the implementation of a subtractive proteomics (SPA) approach, an *in-silico* computational technique, enabling the retrieval of novel targets. Unknown putative proteins, fictitious proteins, and nameless proteins that haven't been thoroughly studied were some of the identified possible therapeutic targets. For *A. niger* (strain CBS 513.88 / FGSC A1513), an SPA method was used. Proteins are deleted at each stage of SPA. Successful collection of the entire proteome from UniProt. Another computational tool, called CD-HIT, was used to recover the duplicated sequences. After retrieving duplicated sequences, necessary genes were gathered from DEG databases. BLASTp was used to find non-homologous matches. These matches provided us with the KEGG pathway for the interaction between *Aspergillus* species with *Homo sapiens. A. niger* druggability testing was done on the proteins that were left over after the pathway analysis. DrugBank databases are used to do druggability assessments. Using the BUSCA and PSORT programmes, the proteins position was predicted. For *A. niger*, we have found 9 new proteins. As a result, the information about these

vital proteins can be exploited to develop new pharmacological targets for *A. niger* as well as for therapeutic purposes.

Conflicts of Interest

The author declares no conflicts of interest.

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