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IN SILICO ANALYSIS OF ASPERGILLUS FLAVUS FUNGAL PROTEINS: STRUCTURAL AND FUNCTIONAL INSIGHTS USING ITS PRIMERS

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ABSTRACT

Aspergillus spp. is a type of fungi that can contaminate and damage various types of food and agricultural products. To better understand the structure and function of proteins, *in silico* research was conducted using Internal Transcribed Spacer (ITS) primers and SWISS Model protein prediction tools. The objective of this study was to identify fungal species using the BLAST method and to analyze the structure and function of proteins from *Aspergillus* spp. The methods used included BLAST for species identification, Web Expaty to translate DNA sequences into proteins, SWISS Models to model protein structures, SAVES to validate protein structures, and STRING to analyze the function of proteins. The results of the BLAST analysis showed that the identified fungal species were *Aspergillus flavus*, *A. tamarii*, and *A. nomius*. Furthermore, the results of translating DNA sequences into proteins using Web Expaty showed that there were three open reading frames with the highest residual values of 119 and 83, while the lowest residual value was 4. Only two of these frames met the protein criteria. Moreover, the results of protein structure modeling using the SWISS Model method produced a fairly accurate *Aspergillus* spp. protein structure model with a validation value of protein structure using ERRAT (SAVES V6.0) of 100%. Additionally, the results of protein function analysis using STRING showed that the *Aspergillus* spp. protein has a function in producing enzymes that play a role in the metabolic process of cells.

Keywords: *Aspergillus flavus*, *in silico*, ITS primer, protein structure, protein function, "SWISS Model".

INTRODUCTION

Aspergillus spp. fungus is a type of fungus that often causes contamination and damage to various agricultural and food products (Gourama & Bullerman, 1995), such as grains, nuts, and processed products (Ayofemi Olalekan Adeyeye, 2020). One of the fungi *Aspergillus* spp. that can contaminate feed ingredients is the species *A. flavus* which can cause aflatoxin pollution (Klich, 2007), which is a harmful substance that can affect human health and livestock (Balina et al, 2018). *A. flavus* is a group of fungi often found in agricultural environments, especially in food crops such as corn, rice, and legumes (Amaike & Keller, 2011). Contamination by *Aspergillus* spp. can

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cause damage to agricultural products and foodstuffs and can cause health problems in humans and farm animals that consume them (Yu et al, 2005). Therefore, research on *Aspergillus* spp. is very important in agriculture to prevent and control contamination of *Aspergillus* spp. on agricultural products and foodstuffs.

Previous studies have highlighted the negative impact of *Aspergillus* spp. on agricultural products and food items. Ramirez et al. (2018) noted that this type of fungus can cause damage to grains, legumes, and processed products. Similarly, Balina et al. (2018) indicated that *A. flavus* can be a major cause of aflatoxin contamination in feed materials, posing risks to livestock and humans who consume them. The significance of research on *A. flavus* is further underscored by the findings of Bhatnagar-Mathur et al. (2015), demonstrating the frequent presence of *A. flavus* in agricultural environments, with a high tendency to infect staple food crops. Therefore, contamination risk

from *A. flavus* not only affects the quality of agricultural products and food items but also poses a serious health risk to consumers. *In silico* research has become a useful tool for studying the structure and function of proteins (Dutta et al, 2018), as it allows for quick and efficient hypothesis testing and analysis (Kirubakaran et al, 2013). In this study, we used an *in silico* technique to predict the structure and function of the *A. flavus* protein by combining the primary use of the Internal Transcribed Spacer (ITS) and the SWISS Model protein prediction tool. This *in silico* approach is essential to understanding this fungus and is beneficial to the food and agriculture industries.

The formulation of the problem of this study was to analyze the BLAST results of these *Aspergillus* spp. sequences, examine the protein structure related to contamination, evaluate the validity and confidence of the protein structure of *Aspergillus* spp. which was found, as well as examining the function of the protein *Aspergillus* spp. associated with contamination of corn feed and other foodstuffs, as well as to understand the structure and function of the *Aspergillus* spp. protein associated with the contamination aforementioned. Therefore, this research focused on the analysis of *Aspergillus* spp. sequence, protein structure analysis, and protein function analysis, with the hope of providing new insights and solutions to the problem of contamination in food.

This study aims to analyze the species *Aspergillus* spp. which often contaminates corn feed and other foodstuffs. This study is expected to provide information about the species *Aspergillus* spp. which contaminates corn feed and other foodstuffs, as well as the structure and function of the protein *Aspergillus* spp. The results of this study are anticipated to contribute to the development of methods for detecting and preventing contamination of *Aspergillus* spp. in corn feed and other foodstuffs.

MATERIALS AND METHODS

Identification of fungus species: This step involved the use of an Internal Transcribed Spacer (ITS) Primer to verify fungus species. This stage of research was carried out by taking DNA samples from fungi and conducting DNA amplification using primary ITS. DNA amplification was performed using the Polymerase Chain Reaction (PCR) technique. This step aimed to verify the species of fungus analyzed. In the PCR technique, DNA samples were placed together with ITS primers (ITS1 5'-TCCGTAGGTGAACCTGCCG-3', and ITS4 5'-TCCTCCGCTTATTGATATGC-3') and polymerase

enzymes, and a heating and cooling process was carried out to start and end the reaction. The result of the amplification process was more DNA sequences associated with ITS. After successful DNA amplification, the obtained DNA sequence was analyzed to verify the fungus species. Sequencing analysis was carried out using bioinformatics software such as BLAST (*Basic Local Alignment Search Tool*) (Lobo, 2008).

Translate DNA Sequencing into Proteins: After the fungus DNA sequence was successfully analyzed, the DNA sequence was then translated into proteins. This step was done using a tool such as the ExPasy Translate Tool (Bashir, 2022). This tool converted the DNA sequence into a protein sequence based on the genetic code (Ayaz et al, 2020). The results of the translation showed the amino acid sequence contained in the fungus protein.

Analysis of protein structure: This stage used the SWISS Model method to predict the protein structure of the fungus *Aspergillus* spp. At this stage, the input of protein sequencing data from ExPasy tools was carried out, and then protein modeling was made using the SWISS Model (Waterhouse et al, 2018). Analysis using the SWISS Model provided information regarding protein domains, protein motifs, and other information related to the structure and function of proteins (Bordoli & Schwede, 2012). The results of the analysis of the structure and function of the protein were re-examined to ensure the validity of the results. Validation was done by comparing the results with a trusted protein database such as UniProt.

Protein Function Analysis: The research method of protein function analysis with STRING involved the use of a database of protein interactions and the analysis of protein networks (Franceschini et al, 2012). In this study, *A. flavus* protein data were used in the STRING database. Protein data were inputted into a STRING database containing information about protein interactions formed based on data from literature databases, scientific publications, and experimental studies (Szkarczyk et al, 2010). Analysis of protein interactions formed using algorithms available on the STRING database. This algorithm generated information about the interaction of formed proteins, the forces of interaction, and their relationship with biological functions. Interpretation of the results of protein interaction analysis was done to obtain information about the biological function of the protein being analyzed and how the protein interaction was related to the biological function found. This method

could be used to analyze the function of proteins and understand how proteins interacted with each other in a network of proteins (Szklarczyk et al, 2023). Using this method, we could gain a better understanding of how proteins were involved in biological processes and their possible effects on health and disease.

RESULTS AND DISCUSSION

Identification of fungus species by BLAST Method:

The results of the fungus species identification using BLAST revealed a similarity of 99.61% with *Aspergillus tamarii*, 98.94% with *Aspergillus flavus*, and 96.33% with *Aspergillus nomius*. These findings suggested that the sample likely belonged to the *Aspergillus* group, with a higher similarity to *A. tamarii* compared to the other two species. *A. tamarii* is recognized for its capability to produce various bioactive compounds (Bose et al, 2019), including phenol, flavonoid, and IAA (Indole-3-Acetic Acid) and its antifungal activity, which have potential applications in Agriculture and food industries. However, it is important to note that *A. tamarii* can also cause

infections in humans and animals (Tam et al, 2014). Similarly, *A. flavus* is known for its production of toxic compounds, such as aflatoxin, which can be harmful to human and animal health (Fouad et al, 2019). Therefore, proper handling measures are necessary to prevent the spread of this species. Moreover, *A. nomius*, another identified species in the sample, can produce aflatoxin and has the potential to become pathogenic in humans and animals (Zain, 2011). Consequently, accurate identification of the fungus species is essential to determine appropriate control measures. In this case, the identification results indicating the highest similarity to *A. tamarii* and *A. flavus* can serve as references for further research on the bioactive compounds produced by these species. Nevertheless, further examinations are required to confirm the presence of specific fungus species in the sample and establish appropriate control measures to prevent its spread.

select all 10 sequences selected		GenBank	Graphics	Distance tree of results	MSA Viewer			
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <i>Aspergillus tamarii</i> strain M24 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.8S...	<i>Aspergillus tamarii</i>	1419	2347	97%	0.0	99.61%	889	ON819577.1
<input checked="" type="checkbox"/> <i>Aspergillus flavus</i> strain Bp5 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1 and 5.8S riboso...	<i>Aspergillus flavus</i>	1343	2094	94%	0.0	98.94%	939	KF221065.1
<input checked="" type="checkbox"/> <i>Aspergillus tamarii</i> strain BPMF17 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1,...	<i>Aspergillus tamarii</i>	1321	2151	99%	0.0	96.86%	897	MF359724.1
<input checked="" type="checkbox"/> <i>Aspergillus tamarii</i> strain MF24 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.8...	<i>Aspergillus tamarii</i>	1310	1952	98%	0.0	96.83%	791	MF359732.1
<input checked="" type="checkbox"/> <i>Aspergillus</i> sp. AQQWD 17 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and internal tran...	<i>Aspergillus</i> sp. Δ...	1297	2462	100%	0.0	96.37%	1303	KP721583.1
<input checked="" type="checkbox"/> <i>Aspergillus</i> sp. isolate ACSIKS_2100168 small subunit ribosomal RNA gene, partial sequence: internal transcribed spa...	<i>Aspergillus</i> sp.	1291	2430	100%	0.0	96.25%	1187	MN597042.1
<input checked="" type="checkbox"/> <i>Aspergillus</i> sp. isolate SS_12 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.8S...	<i>Aspergillus</i> sp.	1286	2440	100%	0.0	96.12%	1303	MT497446.1
<input checked="" type="checkbox"/> <i>Aspergillus flavus</i> isolate 20 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1 and 5.8...	<i>Aspergillus flavus</i>	1273	1946	91%	0.0	98.18%	715	MK120614.1
<input checked="" type="checkbox"/> <i>Aspergillus nomius</i> strain Tur4 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1 and 5.8S ribos...	<i>Aspergillus nomiae</i>	1240	1891	95%	0.0	96.33%	842	KF221090.1
<input checked="" type="checkbox"/> <i>Aspergillus flavus</i> strain Beca_43 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5...	<i>Aspergillus flavus</i>	1234	2105	99%	0.0	96.80%	849	KY234269.1

Figure 1. The results of the fungus species identification using BLAST Method (GenBank)

Translate DNA Sequencing into Proteins: The results obtained from the translation of *Aspergillus* spp. DNA sequences into proteins using the ExPASy web revealed the presence of three open reading frames (ORFs), indicated in red, with residual values of 119 and 83 as the highest, and 4 as the lowest. Proteins are typically categorized if they consist of more than 50 residues (Damodaran & Parkin, 2017). In this case, only two out of the three ORFs met the criteria to be considered as proteins. The identification of an ORF qualifying as a protein may indicate the presence of a gene within the *Aspergillus* DNA sequence. Translating DNA sequences into proteins holds significant importance in molecular

biology research as it allows for a better understanding of protein functions within a specific organism (Gasteiger et al, 2003). Consequently, these findings serve as a foundation for future investigations concerning the *Aspergillus* gene and its potential applications in various fields, such as agriculture and the food industry. Nevertheless, it is crucial to note that these results should undergo further verification using other techniques, such as protein structure analysis, to ensure the functional integrity of the resulting proteins. Additionally, the authenticity and suitability of the *Aspergillus* DNA sequence utilized in this study should be validated about the specific *Aspergillus* species under investigation.



Figure 2. The results of *Aspergillus* spp. DNA sequences into proteins using the ExpAsy web Table 1. The results of Open Reading Frames (ORFs) with residual values from 4 to 119

Open Reading Frame	Residue	Types of Molecules	Code Peptides/Proteins
MNGGPAEATKVQ	12	Peptide	A1
MAGPPKQLRYSKHGWEVGLARNPTLGNDPSVGEPAEGSLPSVGFLASPTSHPCLLYLSCFGGPAIHGRRGLSAPGPRP PETPRTLSDLVKSELIVSVKTFNNGSLGSGIDEERSEMR	119	Protein	A2
MPVRASLLPIKHGLCVGSSSPLRGGRAPKAAAPRPIILERMGLCHPLCRPGRRLPNANQSFRLTSDQVIGIPAEELKHINKAEE	83	Protein	A3
MILP	4	Peptide	A4
MAY	4	Peptide	A5
MAAGGSQPRARARRRRHELCLI	22	Peptide	A6
MDLLVPASMKNAAKCDN	17	Peptide	A7

Protein Structure Modeling with the SWISS Model method: The results of modeling the protein structure of *Aspergillus* spp. using the SWISS Model method shows the presence of a 3D structure of proteins formed (Bordoli & Schwede, 2012). Protein structure modeling is an important method in molecular biology research because it can provide information about the shape and function of proteins in a particular organism (Breda et al, 2007). The results of modeling the protein structure of *Aspergillus* spp. can be used as a basis for conducting further analysis of protein function, including interactions with other molecules, enzymatic activity, and possible drug development. In addition, these results can also help in understanding the relationship between the structure of proteins and their biological activity.

However, keep in mind that the results of modeling protein structures using computational methods such as the SWISS Model still have limitations, especially in terms of accuracy. Therefore, these results still need to be verified using other techniques, such as X-ray crystallography or core magnetic resonance spectroscopy (NMR), to ensure the accuracy of the resulting protein structure. In addition, modeling the protein structure of *Aspergillus* spp. can also provide information about the evolutionary relationship of proteins between different *Aspergillus* species. Modeling the structure of the *Aspergillus* spp. protein can help understand the differences and similarities between different *Aspergillus* proteins so that they can be used as a basis for specific drugs and therapeutic development.



Figure 3. Protein Structure Prediction from Internal Transcribed Spacer (ITS) primer of *Aspergillus flavus* fungus using Protein Prediction from SWISS Model. (Template: A2: 3lb6.1.A A3: 5t7v.1.K)

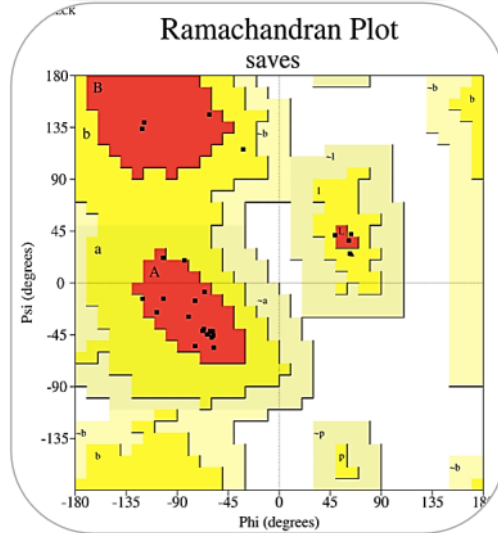
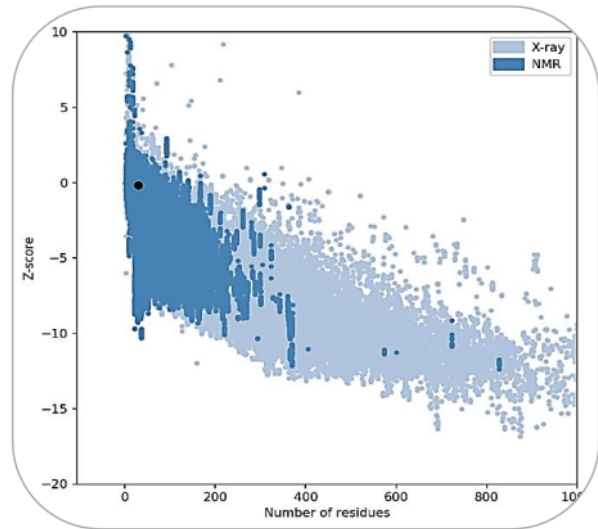
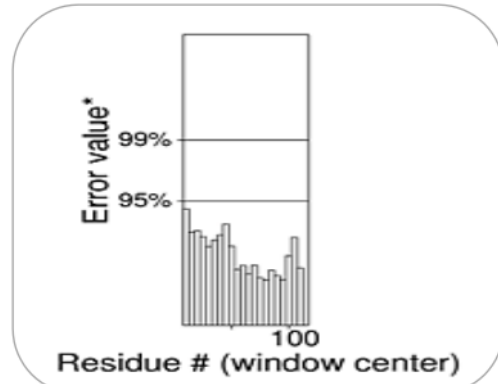
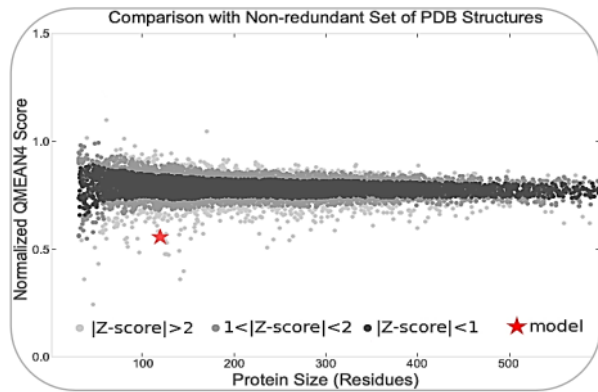
The results of validation of the *Aspergillus* spp. protein structure using SAVES V6.0 shows that the resulting protein structure has a fairly good value in terms of accuracy and quality. One of the parameters used to evaluate the validation of protein structure is the Ramachandran plot, which shows the proportion of amino acids placed in the most favored regions within the protein conformation space (Laskowski et al, 2013). In the Ramachandran plot results obtained, the highest Residual value in most favored regions was 80% for the protein code A2, which indicates that most of the amino acids in the protein structure of *Aspergillus* spp. were placed in the desired area (Lovell et al, 2003). In addition, the validation results using the ERRAT method also show the highest value of 100%, which indicates that the resulting protein structure has high accuracy (Rozario et al, 2021). The results of the validation of the protein structure of *Aspergillus* spp. using SAVES V6.0 is important to ensure that the resulting protein structure is trustworthy and used as a basis for further analysis of protein function and drug development. Validation of protein structure can also help identify and correct parts in the protein structure that may be inaccurate, thereby improving the quality and accuracy of research results.

Table 2. Protein Structure Validation using SAVES V6.0

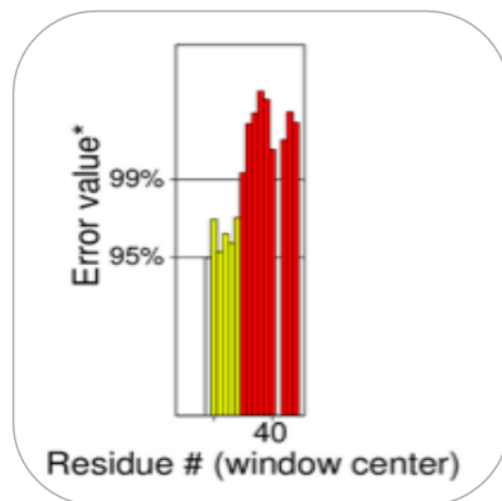
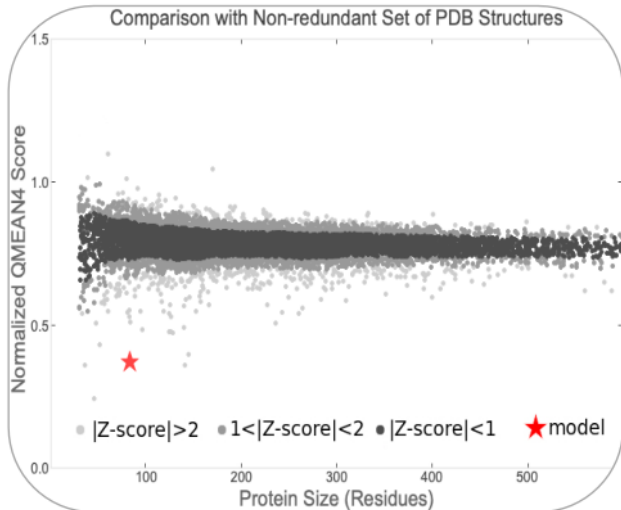
Protein Code	Ramachandran plot				ERRAT quality factor (%)	QMEAN 4 Score	Z Score
	Residu in most favored regions (%)	Residu in additional allowed regions (%)	Residu in generously allowed regions (%)	Residu in disallowed regions (%)			
A2	80.0	20.0	0.0	0.0	100	-1.72	-0.16
A3	75.0	20.0	0.0	5.0	6.6666	-3.27	-3.32

The QMEAN4 Score indicates the overall quality of the protein structure, which is calculated based on geometric values, non-covalent interactions, and consistency of the structure (Benkert et al, 2008). The higher the QMEAN 4 Score, the better the quality of the resulting protein structure (Benkert et al, 2011). In this case, the QMEAN4 Score A2 value of -1.72 versus A3 of -3.27 can be interpreted as a fairly low value, which indicates that the resulting *Aspergillus* spp. protein structure does not have optimal quality. Meanwhile, the Z Score is a parameter used to compare the quality of the resulting protein structure with other similar protein structures, which are calculated based on geometric values and structural consistency. The higher the Z Score value, the better the quality of the resulting protein structure compared to other similar protein structures (Bhattacharya et al, 2007). In this case, a Z Score of -0.16 indicates that the resulting *Aspergillus* spp. protein structure is of relatively good quality compared to other similar protein structures. However, keep in mind that as with the QMEAN 4 Score value, the Z Score value also has limitations and possible errors, so this result still needs to be verified using other protein structure validation techniques.

Model A2



Model A3



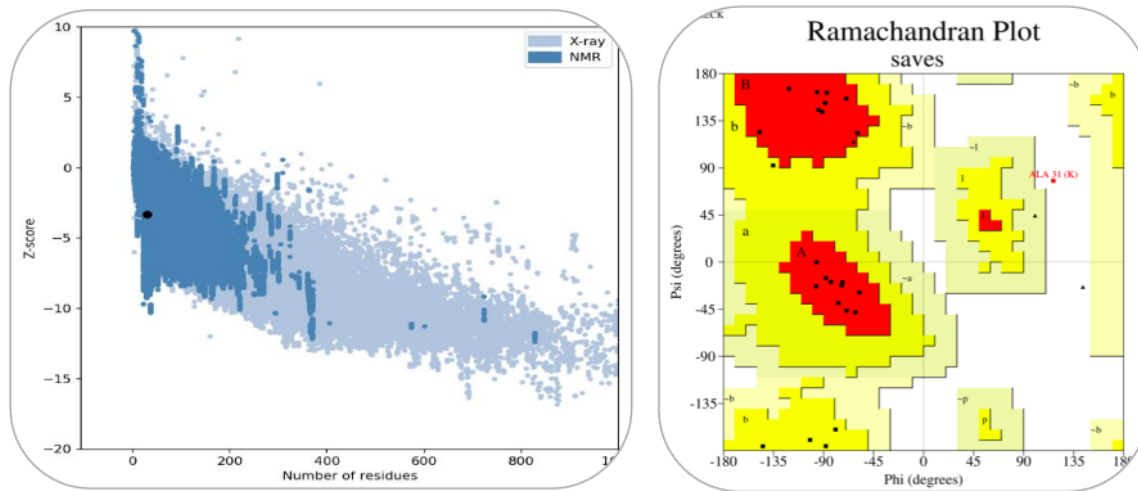


Figure 4. The results of validation of the *Aspergillus* spp. protein structure using SAVES V6.0

Protein Function & Interaction of *Aspergillus Flavus*: *A. flavus* is a type of fungus known to cause damage to agricultural products such as grains and other foodstuffs (Gourama & Bullerman, 1995). This fungus can also produce some toxic substances such as aflatoxins, which are heterocyclic substances and have detrimental effects on human and animal health (Dhanamjayulu et al, 2019). Heterocyclics in aflatoxins have several main functions in the fungus *A. flavus*, including: (1) As a toxic compound: Aflatoxins have high toxic activity and can cause food poisoning in humans and animals, which can cause various health problems such as liver damage, cancer, and reproductive problems (Ogodo & Ugbogu, 2016). (2) As an antioxidant compound: Aflatoxin has antioxidant properties and can help the fungus *A. flavus* survive in a poor environment (Abrar et al, 2013). (3) As an antifungal compound: Aflatoxin can help the fungus *A. flavus* resist attacks from other fungi that seek to interfere with the growth of the fungus *A. flavus* (Bhatnagar-Mathur et al, 2015). Overall, heterocyclic in aflatoxins has an important role in the biology of the fungus *A. flavus*, but it also has detrimental effects on human and animal health. Therefore, it is important to control the production of aflatoxins and ensure that the food products consumed do not contain this substance in harmful quantities.

Organic cyclic compound binding is the process of binding organic chemical compounds that have a benzene ring (Cram & Cram, 1971). In this case, such binding may take place in the fungus *A. flavus*. Organic chemical compounds that bind to the fungus *A. flavus* can affect the biological activity of the fungus (Nguyen et al, 2019). Some organic chemical compounds that can bind to the fungus *A. flavus* include growth regulators,

metabolic regulators, and anti-fungal substances (Hou et al, 2022). The binding of organic chemical compounds to the fungus *A. flavus* can affect the growth and reproduction of fungi, modulate enzyme activity, affect the production of toxic substances such as aflatoxins, and affect the interaction of fungi with their environment. Overall, the binding function of organic chemical compounds to the fungus *A. flavus* is essential for understanding the biology of the fungus and how the fungus interacts with its environment. Further research on the binding of organic chemical compounds to the fungus *A. flavus* can help in controlling the production of aflatoxins and improving the quality of food products.

Nucleic acid binding protein has a very important function in the fungal cells of *A. flavus*. Nucleic acids are important molecules that carry genetic information and play a key role in various biological processes, such as DNA replication, RNA transcription, and protein synthesis (Blackburn, 2006). Nucleic acid binding proteins can bind to nucleic acid molecules, facilitate interaction with enzymes and transcription factors, and influence the biological activity of nucleic acid molecules (Von Hippel et al, 1984). In the fungus *A. flavus*, Nucleic acid binding proteins may play a role in regulating gene activity, affecting growth and reproduction, and modulating the fungus' response to the environment. Studies of Nucleic acid binding proteins in the fungus *A. flavus* can provide information about the mechanisms of gene regulation and how fungi react to their environment. Overall, the function of the Nucleic acid binding protein in the fungus *A. flavus* is essential for understanding the biology of the fungus and how the fungus interacts with its environment. More research on Nucleic acid binding proteins in the fungus

A. flavus can help understand the growth and reproduction mechanisms of fungi, as well as how fungi react to their environment.

RNA binding is a process in which proteins bind to RNA molecules. RNA is an important molecule in cells that plays a key role in biological processes such as transcription, protein synthesis, and gene regulation (Elliott & Ladomery, 2017). RNA protein binding can bind to RNA molecules and facilitate interaction with enzymes and transcription factors (Corley et al, 2020). The function of RNA binding proteins can affect the biological activities of RNA molecules, such as modulating transcription and protein synthesis, affecting RNA stability, and facilitating gene control (Gilsovic et al, 2008). In the fungus, *A. flavus*, protein RNA binding may play an important role in regulating gene activity, modulating responses to the environment, and facilitating growth and reproduction. Studies on protein RNA binding in the fungus *A. flavus* may help understand the mechanisms of gene regulation and how fungi react to their environment. Overall, the function of RNA binding proteins in the fungus *A. flavus* is critical to understanding the biology of the fungus and how the fungus interacts with its environment. More research on protein RNA binding in the fungus *A. flavus* may help in understanding the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

mRNA binding is a process in which proteins bind to mRNA molecules (Dreyfuss et al, 2002). mRNA is an RNA molecule that contains genetic information from DNA and is used as a template for protein synthesis (Alberts et al, 2002). mRNA protein binding can bind to mRNA molecules and facilitate interaction with enzymes and transcription factors (Corley et al, 2020). In the fungus *A. flavus*, mRNA protein binding may play an important role in regulating gene activity, modulating responses to the environment, and facilitating growth and

reproduction. Studies on mRNA protein binding in the fungus *A. flavus* may help understand the mechanisms of gene regulation and how fungi react to their environment. Overall, the function of mRNA protein binding in the fungus *A. flavus* is critical to understanding the biology of the fungus and how the fungus interacts with its environment. More research on mRNA protein binding in the fungus *A. flavus* may help understand the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

Ribosomes are cellular structures that play a key role in the process of protein synthesis (Frank & Spahn, 2006). Ribosomes consist of two subunits, a large subunit and a small subunit, which serve to bind amino acids and form polypeptide chains (Lake, 1981). Proteins that are structural components of ribosomes can play an important role in ensuring the integrity and activity of ribosomes. The function of proteins as structural components of ribosomes can affect the ability of ribosomes to bind to mRNA and facilitate proper protein synthesis (de la Cruz et al, 2015). In the fungus *A. flavus*, proteins that are structural components of ribosomes may play an important role in ensuring the growth and reproduction of fungi. The study of proteins that are structural components of ribosomes in the fungus *A. flavus* can help understand the mechanism of protein synthesis and how the fungus reacts to its environment. Overall, the function of proteins as structural components of ribosomes in the fungus *A. flavus* is essential for understanding the biology of the fungus and how the fungus interacts with its environment. More research on the proteins that are structural components of ribosomes in the fungus *A. flavus* can help in understanding the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

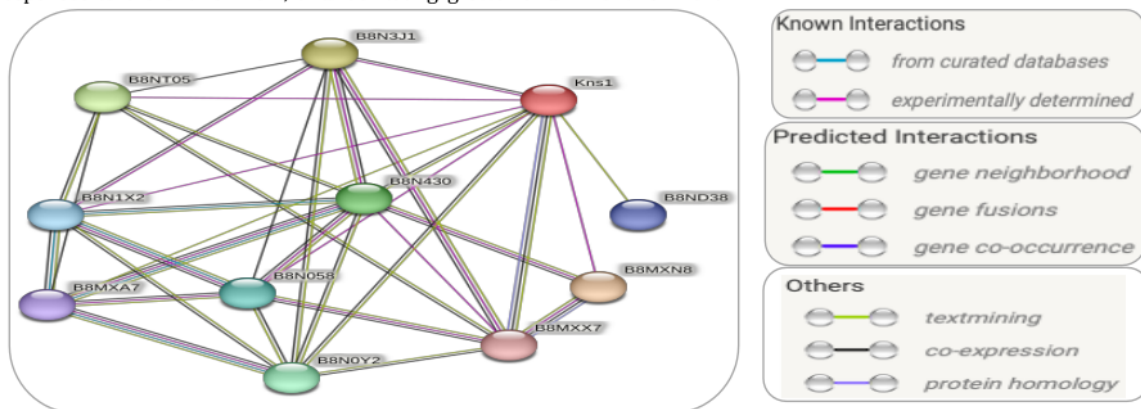


Figure 5: Network of protein-protein interactions visualized with STRING (NCBI taxon-Id: 332952)

● B8MXN8	Serine/threonine protein kinase, putative
● B8N3J1	Small nucleolar ribonucleoprotein complex subunit Utp14, putative
● B8NT05	G-protein complex beta subunit CpcB
● B8N430	Nuclear mRNA splicing factor, putative
● B8N0Y2	G-patch domain protein (TFIP11), putative
● B8N058	PWI domain mRNA processing protein, putative
● B8N1X2	Pre-RNA splicing factor Srp2, putative
● B8ND38	CaaX prenyl proteinase Rce1
● B8MXA7	Small nuclear ribonucleoprotein E; Associated with the spliceosome snRNP U1, U2, U4/U6 and U5.
● B8MXX7	Casein kinase, putative; Belongs to the protein kinase superfamily.

Table 3: Molecular Function, Biological Process, Cellular Component, and Subcellular Localization Classification

Molecular Function	Biological Process	Cellular Component	Subcellular Localization
<ul style="list-style-type: none"> • Heterocyclic compound binding • Organic cyclic compound binding • Nucleic acid binding • RNA binding • mRNA binding • Structural constituent of ribosome • rRNA binding • snoRNA binding 	<ul style="list-style-type: none"> • mRNA splicing, via spliceosome • RNA processing • Gene expression • Macromolecule metabolic process • Cellular nitrogen compound metabolic process • Nitrogen compound metabolic process • Primary metabolic process • Cellular metabolic process 	<ul style="list-style-type: none"> • Spliceosomal complex • Ribonucleoprotein complex • Nucleus • Intracellular membrane-bounded organelle 	<ul style="list-style-type: none"> • U2 snRNP • U2-type prespliceosome • U2-type spliceosomal complex • Spliceosomal complex • Ribonucleoprotein complex • Nucleus • Protein-containing complex • Intracellular membrane-bounded organelle • Intracellular • Cellular anatomical entity

CONCLUSION

In silico research was conducted to understand the structure and function of proteins of *Aspergillus* spp. The results of the BLAST analysis showed the presence of three species of fungi, namely *Aspergillus flavus*, *A. tamarii*, and *A. nomius*. Translating DNA sequences into proteins using Web Exspasy showed that only two proteins met the criteria. Protein structure modeling using SWISS Model resulted in a fairly accurate *Aspergillus* spp. protein structure model with protein structure validation values using ERRAT (SAVES V6.0) of 100%. Analysis of protein function using STRING shows that *Aspergillus* spp. protein has a function in producing enzymes that play a role in the metabolic process of cells. Thus, this study provides important information about the structure and function of the protein *Aspergillus* spp. which can be used to improve understanding of contamination and damage to food and agricultural products by this type of fungus.

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Samharinto Soedijo	: Provided specialized insights and edited the manuscript
Yusriadi Marsuni	: Offered biochemical insights and managed references
Muslimin Sepe	: Participated in statistical analysis and managed data integrity
Muhammad I. Pramudi	: Identified bioinformatics resources and contributed to materials and methods
Ismed S. Budi	: Provided a microbiological perspective and contributed to conclusions.
Dewi Fitriyanti	: Participated in data analysis, and assisted in manuscript preparation
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Noor Aidawati	: Assisted in experimental design and contributed to data interpretation
Helda O. Rosa	: Drafted the introduction, handled revisions, and responded to feedback.

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