COVER LETTER

Dear **Editor-in-Chief,**

I am enclosing herewith a manuscript,

Title:

In Silico Analysis of *Aspergillus flavus* Fungal Proteins: Structural and Functional Insights Using ITS Primers

Author(s) name:

[1]Mariana Mariana, [2]Saipul Abbas, [3]Salamiah Salamiah, [4]Samharinto Samharinto, [5]Yusriadi Marsuni, [6]Muslimin Sepe, [7]Muhammad Indar Pramudi, [8]Ismed Setya Budi, [9]Dewi Fitriyanti, [10]Elly Liestiany, [11]Lyswiana Aphrodyanti

Address

(Fill in your institution's name and address, your personal cell phone and email)

I. First author:

Name : Mariana Mariana
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : mariana@ulm.ac.id
Celluler Phone : +6285732719460

II. Second author and Corresponding author:

Name : Saipul Abbas
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : saipul.abbas@ulm.ac.id
Celluler Phone : +6285310104906

III. Third author:

Name : Salamiah Salamiah
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : salamiah@ulm.ac.id
Celluler Phone : +628115011749

IV. Fourth author:

Name : Samharinto Samharinto
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : samharinto@ulm.ac.id

Formatted: Font: (Default) Times New Rom
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: 12 pt
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom Font color: Auto, Character scale: 100%
Formatted: Font: (Default) Times New Rom 12 pt

Celluler Phone : +6285249229863

V. Fifth author:
Name : Yusriadi Marsuni
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : yusriadimarsuni@ulm.ac.id
Celluler Phone : +628125068762

VI. Sixth author:
Name : Muslimin Sepe
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : muslimins@ulm.ac.id
Celluler Phone : +6281331258610

VII. Seventh author:
Name : Muhammad Indar Pramudi
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : muhamadindarpramudi@ulm.ac.id
Celluler Phone : +6281351268811

VIII. Eighth author:
Name : Ismed Setya Budi
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : ibudi@ulm.ac.id
Celluler Phone : +6281933753340

IX. Ninth author:
Name : Dewi Fitriyanti
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : dewifitriyanti@ulm.ac.id
Celluler Phone : +628970073639

X. Tenth author:
Name : Elly Liestiany
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : elly.liestiany@ulm.ac.id
Celluler Phone : +6287816006236

Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt

xi. Eleventh author:

Name : Lyswiana Aphrodyanti
Afiliation : Lambung Mangkurat University
Address. : Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia
E-mail : lyswianaaphrodyanti@ulm.ac.id
Celluler Phone : +6281348208441

Type of article : a. Research Paper b. Short Communication c. Review
(Please choose according to your article by underlining the available options)

For publication in the Pakistan Journal of Phytopathology

Novelty:
(state your claimed novelty of the findings versus current knowledge)
There is no in-depth information available about protein structure of *Aspergillus flavus* in South Kalimantan, Indonesia

Statements:
This manuscript has not been published elsewhere, accepted for publication elsewhere, or under consideration for publication elsewhere (including web hosting) either by me or any of my co-authors; and my institute's (Lambung Mangkurat University) representative is fully aware of this submission. The author (s) has read and agreed to the Ethical Guidelines.

Contribution of Authors:
All authors contributed equally to this research.

Place and date:
2023-11-26

Sincerely yours,
(fill in your name, no need for a scanned autograph)
Saipul Abbas

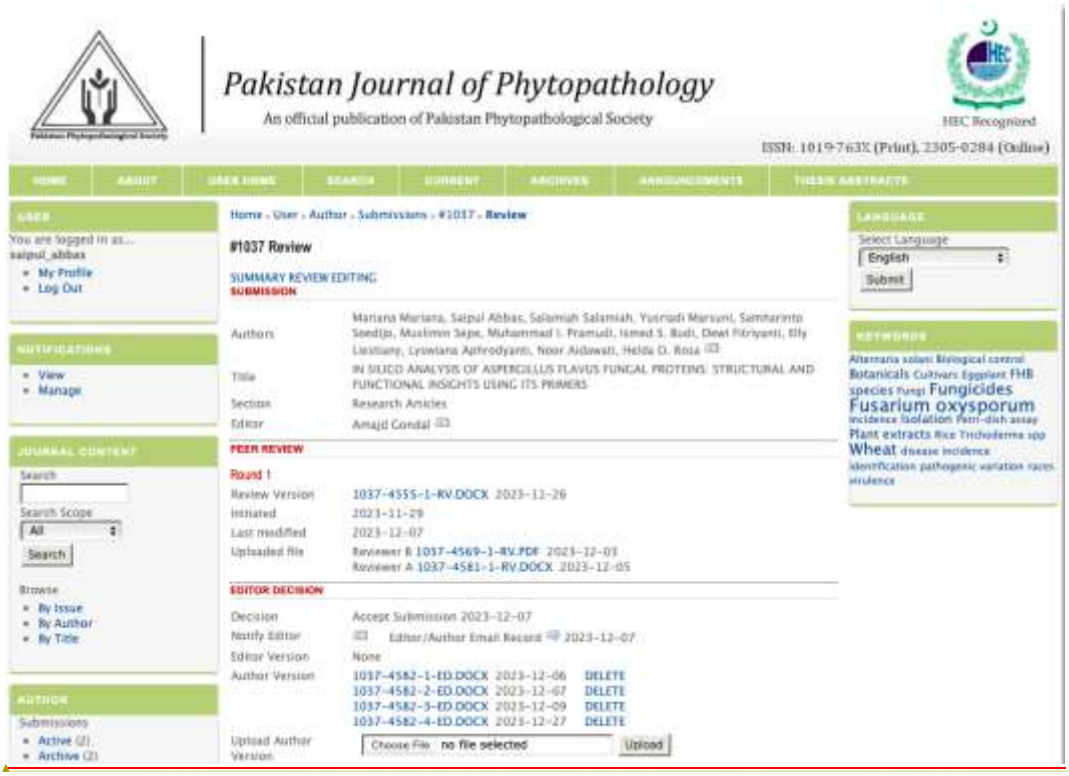
1. Submitted to the journal "Pakistan Journal of Phytopathology" (28-06-2023)
2. Review Version (26-11-2023)

Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Rom 12 pt
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font color: Auto
Formatted: Font color: Auto

3. Initiated (29-11-2023)
4. First revision: Accepted with minor revision (03-12-2023)
5. First revision submitted (06-12-2023)
6. Last Modified (07-12-2023)
7. Second revision: Minor revisions (05-12-2023)
8. Second revision submitted (07-12-2023)
9. Accept Submission (07-12-2023)
10. Copyeditting (27-12-2023)

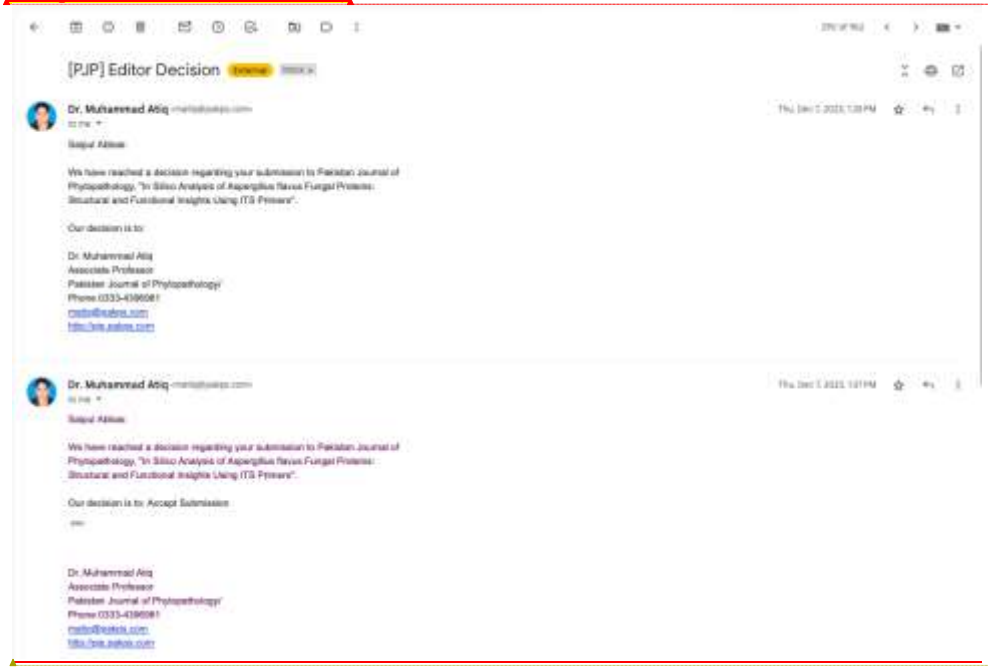
Document Search: https://www.scopus.com/record/display.uri?eid=2-s2.0-85184760451&origin=resultslist

https://www.pjp.pakps.com/index.php/PJP/article/view/1037



Formatted: Font color: Auto
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font color: Auto
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font: 11 pt, Indonesian
Formatted: Font color: Auto
Formatted: Font: 11 pt, Indonesian
Formatted: Font color: Auto, Indonesian
Formatted: Font color: Auto, Indonesian
Formatted: Font color: Auto, Indonesian
Formatted: Underline, Font color: Blue
Formatted: Underline, Font color: Blue
Formatted: Left, Indent: Left: 0,63 cm, First line: 0 cm, Line spacing: single
Formatted: Font: (Default) Times New Roman, Underline, Not Highlight
Formatted: Font: (Default) Times New Roman

Accept Submission (07-12-2023)



Copyeditting (27-12-2023)

Formatted: Highlight

Formatted: Font: (Default) Times New Rom Highlight

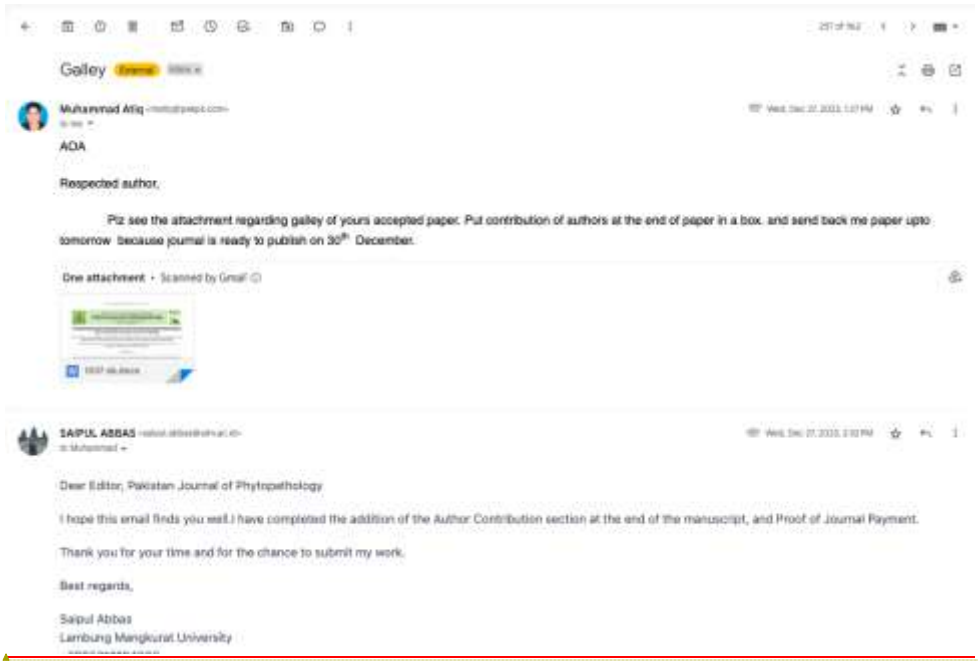
Formatted: Font: Not Highlight

Formatted: Left, Indent: Left: 0,63 cm, Firs line: 0 cm, Line spacing: single

Formatted: Font: (Default) Times New Rom

Formatted: Highlight

Formatted: Left, Indent: Left: 0,5 cm, Hanging: 0,25 cm



Layout (29-12-2023)

Published (30-12-2023)

Formatted: Font: (Default) Times New Rom

Formatted: Highlight

Formatted: Font: (Default) Times New Rom

Formatted: Check spelling and grammar

Formatted: Indent: First line: 0 cm, Line spacing: single

Formatted: Highlight

Formatted: Check spelling and grammar

Formatted: Indent: First line: 0 cm, Line spacing: single

AUTHOR FEES

Article Publication Fee	Paid December 29, 2023 - 07:28 AM

STATUS

Status	Published Vol 35, No 2 (2023): Pak. J. Phytopath.
Initiated	2023-12-30
Last modified	2024-01-02

https://www.pjp.pakps.com/index.php/PJP/article/view/1037


Pakistan Journal of Phytopathology

An official publication of Pakistan Phytopathological Society

HEC Recognized

ISSN: 1019-763X (Print), 2305-0284 (Online)

Home › Vol 35, No 2 (2023) › Mariana

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

Mariana Mariana, Saipul Abbas, Salamiah Salamiah, Yusriadi Marsuni, Samharinto Soedijo, Muslimin Sepe, Muhammad I. Pramudi, Ismed S. Budi, Dewi Fitriyanti, Dly Liestiany, Lyswiana Aphrodyanti, Noor Aidawati, Helda O. Rosa

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 Expasy 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 Expasy 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 modelling 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.

Formatted: Font: (Default) Times New Rom

Formatted: Check spelling and grammar

Formatted: Font: Italic, Highlight

Formatted: Centered, Indent: First line: 0 c Line spacing: single

Formatted: Font: Italic, Check spelling and grammar

Formatted: Font: (Default) Times New Rom

Formatted: Check spelling and grammar

Formatted: Indent: First line: 0 cm, Line spacing: single

First revision: Accepted with minor revision (03-12-2023)

In Silico Analysis of *Aspergillus flavus* Fungal Proteins: Structural and Functional Insights Using ITS Pr

Mariana Mariana, Saipul Abbas*, Salamiah Salamiah, Samharinto Samharinto, Yusri
Muhammad Indar Pramudi, Ismed Setya Budi, Dewi Fitriyanti, Elly Liestiany,

Department of Plant Protection, Faculty of Agriculture, Lambung Mangkurat Univers ity. Jl.Jend. A. Yani Km.36
Banjarbaru. South Kalimantan 70714 Indonesia

*Corresponding author: Email: saipul.abbas@ulm.ac.id

ABSTRACT

Aspergillus ~~flavus~~ is a type of ~~fungus~~ that can contaminate and damag and agricultural products. To better understand the structure and functi research was conducted using Internal Transcribed Spacer (ITS) prim protein prediction tools. The objective of this study was to identify fu BLAST method and to analyze the structure and function of proteins fro methods used included BLAST for species identification, Web Exp sequences into proteins, SWISS Models to model protein structures, SA structures, and STRING to analyze the function of proteins. The results of the BLAST analysis showed that the identified fungal species were *Aspergillus flavus*, *Aspergillus tamarii*, and *Aspergillus nomius*. Furthermore, the results of translating DNA sequences into proteins using Web Expasy 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, p Model

INTRODUCTION

Aspergillus spp fungus is a type of fungus that often caus s contamination and damage to various agricultural and food products (Gourama & Bulle grains, nuts, and processed products (Ayofemi Olalekan Adeyeye, 20 Aspergillus ~~spp~~ that can contaminate feed ingredients is *the species A* can cause aflatoxin pollution (Klich, 2007), which is a harmful substance health and livestock (Balina et al, 2018). *~~Aspergillus~~ flavus* is a group agricultural environments, especially in food crops such as corn, rice, a Keller, 2011). Contamination by *Aspergillus spp.* can cause damage t and foodstuffs and can cause health problems in humans and farm them (Yu et al, 2005). Therefore, research on *Aspergillus spp.* is very i to prevent and control contamination of *Aspergillus spp.* on agricultural products and foodstuffs.

nsakr 2023-12-03 08:50:03
in italic, please.

nsakr 2023-12-03 08:51:27
spp.

nsakr 2023-12-03 08:54:35
not in italic

nsakr 2023-12-03 08:55:50
add "."

nsakr 2023-12-03 09:11:16

nsakr 2023-12-03 09:11:45
.

Formatted: Font: (Default) Times New Rom
Font color: Black, Highlight

Formatted: Normal, Indent: Left: 0,63 cm, bullets or numbering

Formatted: Font: (Default) Times New Rom

Formatted: Font: (Default) Calibri, 14 pt, B

Formatted: Font: (Default) Calibri, 12 pt

Formatted: Font: 12 pt

Formatted: Font: (Default) Calibri, 12 pt

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.

Formatted: Font: (Default) Calibri, 12 pt

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 s[illegible] contribute to the development of methods for detecting and prever[illegible] Aspergillus spp. in corn feed and other foodstuffs.

nsakr
2023-12-03 09:14:48
---
please select another word in place of it is used in the previous sentence.

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'-TCCGTAGGTGAACCTGCGG-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, *Aspergillus 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 (Szklarczyk 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

Formatted: Font: (Default) Calibri, 12 pt, Bo

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 *Aspergillus tamarii* compared to the other two species. *Aspergillus tamarii* is recognized for its capability to produce various bioactive compounds (Bose et al, 2019), including phenol, flavonoid and IAA and its antifungal activity, applications in the Agriculture and food industries. However, it is i *Aspergillus tamarii* can also cause infections in humans and anima Similarly, *Aspergillus flavus* is known for its production of toxic compo which can be harmful to human and animal health (Fouad et al, 201 handling measures are necessary to prevent the spread of this species. *nomius*, another identified species in the sample, can produce aflatox become pathogenic in humans and animals (Zain, 2011). Co identification of the fungus species is essential to determine appropriate control measures. In this case, the identification results indicating the highest similarity to *Aspergillus tamarii* and *Aspergillus 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.

nsakr
2023-12-03 09:17:35
---
please clarify this abbreviation

Formatted: Font: (Default) Calibri, 12 pt

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.

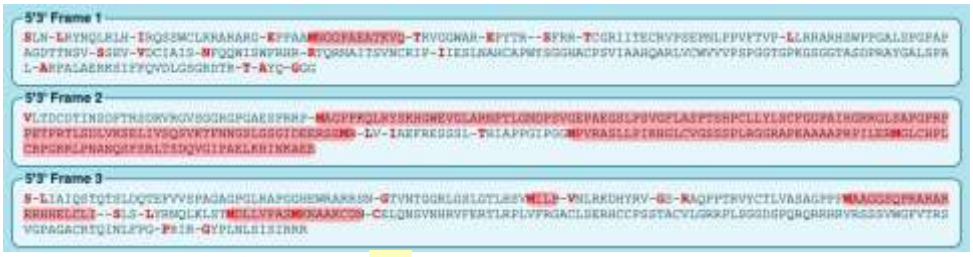


Figure 2: The results of *Aspergillus spp* DNA sequences into proteins using the ExPASy web

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
MAGPPKQLRYSKHGWEVGLARNPTLGND PSVGEPAEGSLPSVGFLASPTSHPCLLYLSC FGGPAIHGRRGLSAPGPRPPETPRTLSDLV KSELIVSQSVKTFNNGSLGSGIDEERSEMR	119	Protein	A2
MPVRASLLPIKHGLCVGSSSPLRGGRAPKA AAAPRPILERMGLCHPLCRPGRRLPNANQS FSRLTSDQVGIPAELKHINKAEE	83	Protein	A3
MILP	4	Peptide	A4
MAY	4	Peptide	A5
MAAGGSQPRARARRRHHELCLI	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 show 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 it can be used as a basis for specific drug and therapeutic development.

Formatted: Font: (Default) Calibri, 12 pt

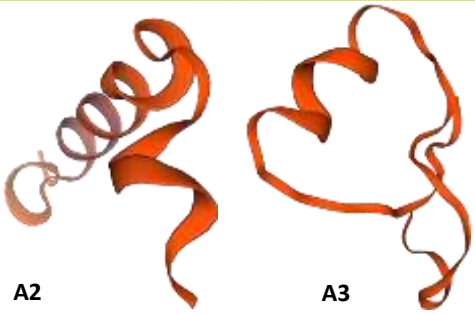


Figure 3: Protein Structure Prediction from Internal Transcribed Spacer (ITS) primer *of Aspergillus flavus* fungus using Protein Prediction from SWISS Model. (Template: A2: 3lb6.1.A A3: 5t7v.1.K)

The results of validation of the *Aspergillus spp* protein structure using SAVES V6.0 show 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 are 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.

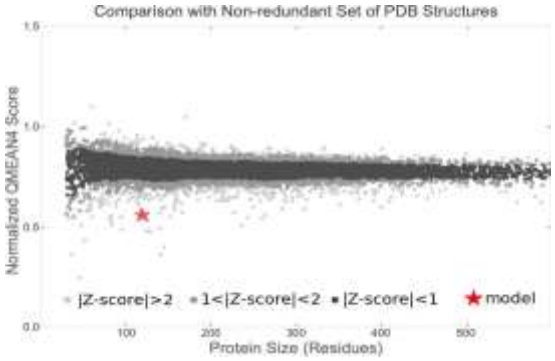
The QMEAN4 Score indicates the overall quality of the protein structure, which is calculated based on geometric values, non-covalent interactions, and consistency of the structure (Benkert et al, 2008). The higher the QMEAN 4 Score, the better the quality of the resulting protein structure (Benkert et 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.

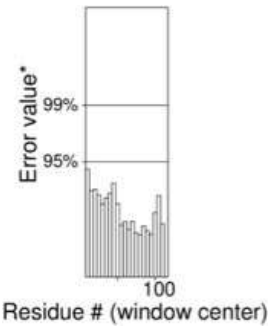
Table 2: Protein Structure Validation using SAVES V6.0

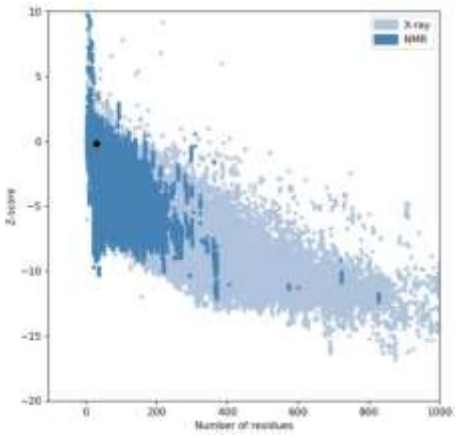
Protein Code	Ramachandran plot				ERRAT quality factor (%)	QMEAN 4 Score	Z Score
	Residu in most favored regions (%)	Residu in additional allowed regions (%)	Residu in generously allowed regions (%)	Residu in disallowed regions (%)			
A2	80.0	20.0	0.0	0.0	100	-1.72	-0.16
A3	75.0	20.0	0.0	5.0	6.6666	-3.27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

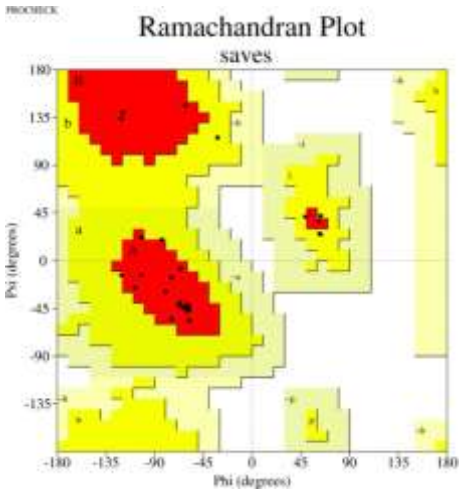
Model A2

Formatted: Font: (Default) Calibri, 12 pt



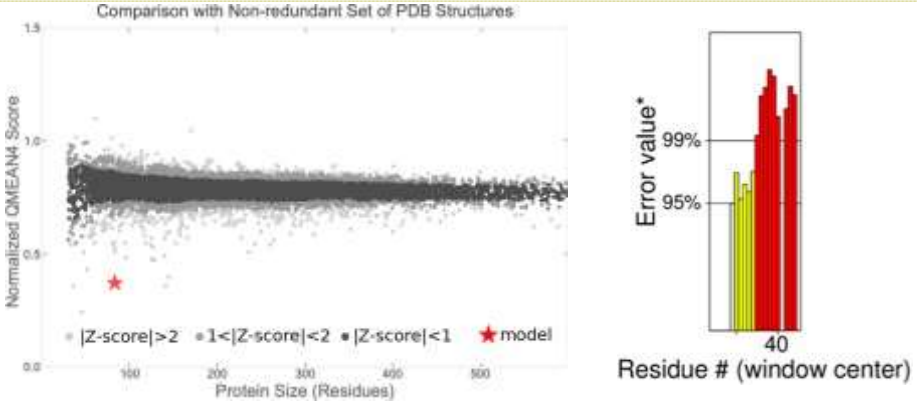






Model A3

Formatted: Font: (Default) Calibri, 12 pt



Formatted: Font: (Default) Calibri, 12 pt

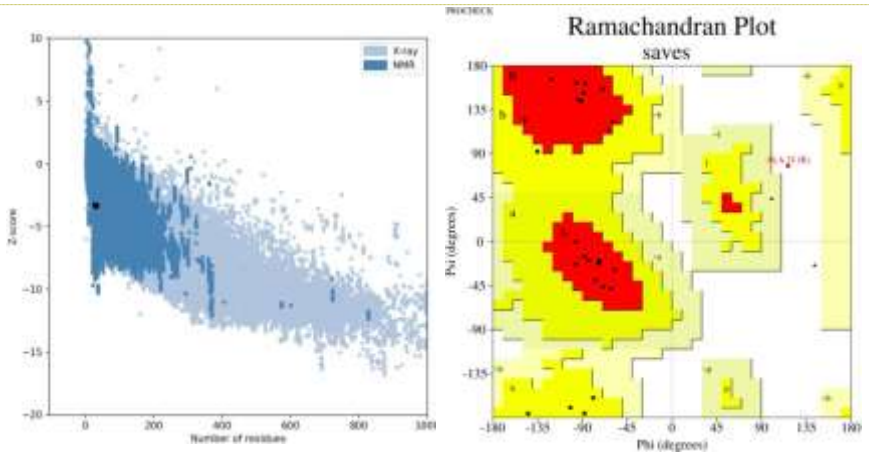


Figure 4: The results of validation of the *Aspergillus spp* protein structure using SAVES V6.0

Protein Function & Interaction of *Aspergillus Flavus*

Aspergillus 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 *Aspergillus 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 *Aspergillus flavus* survive in a poor environment (Abrar et al, 2013). (3) As an antifungal compound: Aflatoxin can help the fungus *Aspergillus flavus* resist attacks from other fungi that seek to interfere with the growth of the

fungus *Aspergillus flavus* (Bhatnagar-Mathur et al, 2015). Overall, heterocyclic in aflatoxins has an important role in the biology of the fungus *Aspergillus 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 *Aspergillus flavus*. Organic chemical compounds that bind to the fungus *Aspergillus flavus* can affect the biological activity of the fungus (Nguyen et al, 2019). Some organic chemical compounds that can bind to the fungus *Aspergillus flavus* include growth regulators, metabolic regulators, and anti-fungal substances (Hou et al, 2022). The binding of organic chemical compounds to the fungus *Aspergillus 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 *Aspergillus 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 *Aspergillus 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 Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus 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, *Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus 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 Aspergillus 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 *Aspergillus 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 *Aspergillus 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 Aspergillus 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 Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus flavus* can help in understanding the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

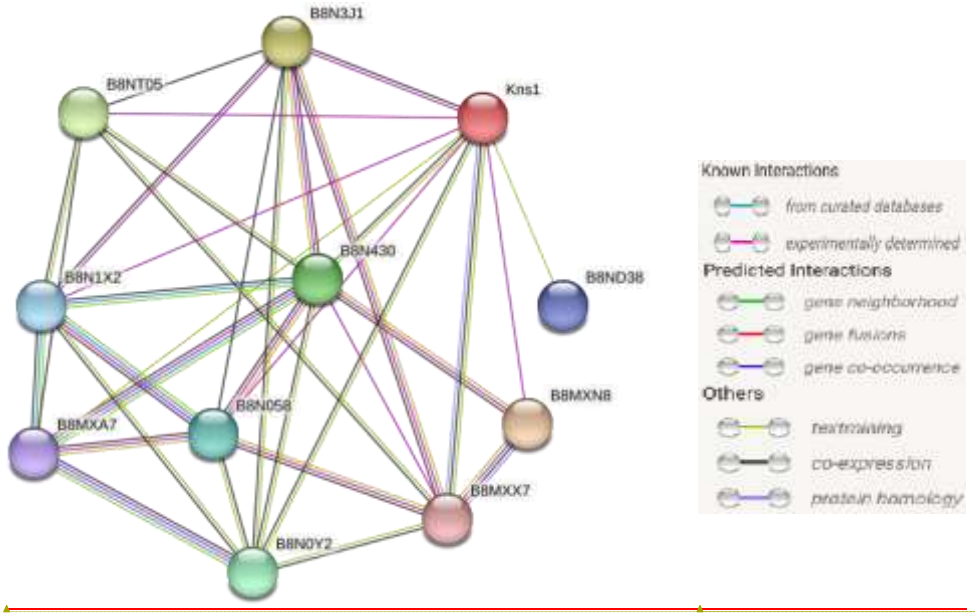


Figure 5: Network of protein-protein interactions visualized with STRING (NCBI taxon-Id: 332952)

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	Sucellular Localization
• Heterocyclic compound binding • Organic cyclic compound binding	• mRNA splicing, via spliceosome • RNA processing • Gene expression	• Spliceosomal complex • Ribonucleoprotein complex • Nucleus	• U2 snRNP • U2-type prespliceosome • U2-type spliceosomal complex

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Raised by 42,5 pt

Formatted: Font: (Default) Calibri, 12 pt

• Nucleic acid binding • RNA binding • mRNA binding • Structural constituent of ribosome • rRNA binding • snoRNA binding	• Macromolecule metabolic process • Cellular nitrogen compound metabolic process • Nitrogen compound metabolic process • Primary metabolic process • Cellular metabolic process	• Intracellular membrane-bounded organelle	• 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, *Aspergillus tamarii*, and *Aspergillus nomius*. Translating DNA sequences into proteins using Web Expasy 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.

Acknowledgement

A thank you to PT. Arutmin Indonesia Tambang Satui and Agreement/Contract Number: 39/UN8.1.23/PP/2022 through the Matching Fund program "Kedaireka" for financial support for this research activity, and all parties who have helped in the success of this research. All the help and support provided means a lot to us. Thanks.

Reference

Ayaz, A., Zaman, W., Saqib, S., Ullah, F., & Mahmood, T. (2020). Phylogeny and diversity of Lamiaceae based on rps14 gene in Pakistan. *Genetika*, *52*(2), 435-452. https://doi.org/10.2298/GENSR2002435A

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). From DNA to RNA. In *Molecular Biology of the Cell. 4th edition*. Garland Science.

Abrar, M., Anjum, F. M., Butt, M. S., Pasha, I., Randhawa, M. A., Saeed, F., & Waqas, K. (2013). Aflatoxins: biosynthesis, occurrence, toxicity, and remedies. *Critical reviews in food science and nutrition*, *53*(8), 862-874. https://doi.org/10.1080/10408398.2011.563154

Ayofemi Olalekan Adeyeye, S. (2020). Aflatoxigenic fungi and mycotoxins in food: a review. *Critical reviews in food science and nutrition*, *60*(5), 709-721. https://doi.org/10.1080/10408398.2018.1548429

Amaike, S., & Keller, N. P. (2011). Aspergillus flavus. *Annual review of phytopathology*, *49*, 107-133. https://doi.org/10.1146/annurev-phyto-072910-095221

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating Aspergillus flavus and aflatoxin contamination in crops. *Plant science*, *234*, 119-132. https://doi.org/10.1016/j.plantsci.2015.02.009

Bhattacharya, A., Tejero, R., & Montelione, G. T. (2007). Evaluating protein structures determined by structural genomics consortia. *Proteins: Structure, Function, and Bioinformatics*, *66*(4), 778-795. https://doi.org/10.1002/prot.21165

Blackburn, G. M. (Ed.). (2006). *Nucleic acids in chemistry and biology*. Royal Society of Chemistry.

Breda, A., Valadares, N. F., de Souza, O. N., & Garratt, R. C. (2007). Protein structure, modelling and applications. In *Bioinformatics in tropical disease research: a practical and case-study approach [Internet]*. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK6824/

Benkert, P., Tosatto, S. C., & Schomburg, D. (2008). QMEAN: A comprehensive scoring function for model quality assessment. *Proteins: Structure, Function, and Bioinformatics*, *71*(1), 261-277. https://doi.org/10.1002/prot.21715

Benkert, P., Biasini, M., & Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, *27*(3), 343-350. https://doi.org/10.1093/bioinformatics/btq662

Bordoli, L., & Schwede, T. (2012). Automated protein structure modeling with SWISS-MODEL Workspace and the Protein Model Portal. *Homology Modeling: Methods and Protocols*, 107-136. https://doi.org/10.1007/978-1-61779-588-6_5

Bose, P., Gowrie, S. U., & Chathurdevi, G. (2019). Optimization of Culture Conditions for Growth and Production of Bioactive Metabolites by Endophytic Fungus-Aspergillus tamarii. *International Journal of Pharmacy and Biological Sciences*, *9*(2), 469-478. https://doi.org/10.21276/ijpbs.2019.9.2.60

Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, *6*(2), e555685. https://doi.org/10.19080/JDVS.2018.06.555685

Bashir, S., Janiad, S., Zeeshan, A., Farah, N., Mehboob, S., Butt, H. I., & Qari, S. H. (2022). In Silico Analysis and Function Prediction of Sr22 Gene Product as Stem Rust Resistant Protein. *Annals of the Romanian Society for Cell Biology*, *26*(01), 258-262. http://annalsofrscb.ro

Cram, D. J., & Cram, J. M. (1971). Cyclophane chemistry: bent and battered benzene rings. *Accounts of Chemical Research*, *4*(6), 204-213. https://doi.org/10.1021/ar50042a003

Corley, M., Burns, M. C., & Yeo, G. W. (2020). How RNA-binding proteins interact with RNA: molecules and mechanisms. *Molecular cell*, *78*(1), 9-29. https://doi.org/10.1016/j.molcel.2020.03.011

de la Cruz, J., Karbstein, K., & Woolford Jr, J. L. (2015). Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo. *Annual review of biochemistry*, *84*, 93-129. https://doi.org/10.1146/annurev-biochem-060614-033917

Dhanamjayulu, P., Boga, R. B., & Mehta, A. (2019). Inhibition of aflatoxin B1 biosynthesis and down regulation of aflR and aflB genes in presence of benzimidazole derivatives without impairing the growth of Aspergillus flavus. *Toxicon*, *170*, 60-67. https://doi.org/10.1016/j.toxicon.2019.09.018

Damodaran, S., & Parkin, K. L. (2017). Amino acids, peptides, and proteins. In *Fennema's food chemistry* (pp. 235-356). CRC Press.

Dreyfuss, G., Kim, V. N., & Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages they carry. *Nature reviews Molecular cell biology*, *3*(3), 195-205. https://doi.org/10.1038/nrm760

Dutta, B., Banerjee, A., Chakraborty, P., & Bandopadhyay, R. (2018). In silico studies on bacterial xylanase enzyme: Structural and functional insight. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 749-756. https://doi.org/10.1016/j.jgeb.2018.05.003

Elliott, D., & Ladomery, M. (2017). *Molecular biology of RNA*. Oxford University Press.

Frank, J., & Spahn, C. M. (2006). The ribosome and the mechanism of protein synthesis. *Reports on Progress in Physics*, *69*(5), 1383. https://doi.org/10.1088/0034-4885/69/5/R03

Fouad, A. M., Ruan, D., El-Senousey, H. K., Chen, W., Jiang, S., & Zheng, C. (2019). Harmful effects and control strategies of aflatoxin b1 produced by Aspergillus flavus and Aspergillus parasiticus strains on poultry. *Toxins*, *11*(3), 176. https://doi.org/10.3390/toxins11030176

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., & Jensen, L. J. (2012). STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic acids research*, *41*(D1), D808-D815. https://doi.org/10.1093/nar/gks1094

Glisovic, T., Bachorik, J. L., Yong, J., & Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. *FEBS letters*, *582*(14), 1977-1986. https://doi.org/10.1016/j.febslet.2008.03.004

Gourama, H., & Bullerman, L. B. (1995). Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food protection*, *58*(12), 1395-1404. https://doi.org/10.4315/0362-028X-58.12.1395

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic acids research*, *31*(13), 3784-3788. https://doi.org/10.1093/nar/gkg563

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, *47*, 101716. https://doi.org/10.1016/j.fbio.2022.101716

Kirubakaran, P., Karthikeyan, M., Singh, K. D., Nagamani, S., & Premkumar, K. (2013). In silico structural and functional analysis of the human TOPK protein by structure modeling and molecular dynamics studies. *Journal of molecular modeling*, *19*, 407-419. https://doi.org/10.1007/s00894-012-1566-1

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, *8*(6), 713-722. https://doi.org/10.1111/j.1364-3703.2007.00436.x

Laskowski, R. A., Furnham, N., & Thornton, J. M. (2013). The Ramachandran plot and protein structure validation. In *Biomolecular forms and functions: a celebration of 50 years of the ramachandran map* (pp. 62-75). https://doi.org/10.1142/9789814449144_0005

Lake, J. A. (1981). The ribosome. *Scientific American*, *245*(2), 84-97. https://doi.org/10.1038/scientificamerican0881-84

Lovell, S. C., Davis, I. W., Arendall III, W. B., De Bakker, P. I., Word, J. M., Prisant, M. G., ... & Richardson, D. C. (2003). Structure validation by Cα geometry: φ, ψ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics*, *50*(3), 437-450. https://doi.org/10.1002/prot.10286

Lobo, I. (2008). Basic local alignment search tool (BLAST). *Nature Education*, *1*(1).

Nguyen, H. N., Chaves-Lopez, C., Oliveira, R. C., Paparella, A., & Rodrigues, D. F. (2019). Cellular and metabolic approaches to investigate the effects of graphene and graphene oxide in the fungi Aspergillus flavus and Aspergillus niger. *Carbon*, *143*, 419-429. https://doi.org/10.1016/j.carbon.2018.10.099

Ogodo, A. C., & Ugbogu, O. C. (2016). Public health significance of aflatoxin in food industry—A review. *European Journal of Clinical and Biomedical Sciences*, *2*(5), 51-58. https://doi.org/10.11648/j.ejcbs.20160205.14

Rozario, L. T., Sharker, T., & Nila, T. A. (2021). In silico analysis of deleterious SNPs of human MTUS1 gene and their impacts on subsequent protein structure and function. *PloS one*, *16*(6), e0252932. https://doi.org/10.1371/journal.pone.0252932

Ramirez, M. L., Cendoya, E., Nichea, M. J., Zachetti, V. G. L., & Chulze, S. N. (2018). Impact of toxigenic fungi and mycotoxins in chickpea: A review. *Current Opinion in Food Science*, *23*, 32-37. https://doi.org/10.1016/j.cofs.2018.05.003

Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., ... & Mering, C. V. (2010). The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic acids research*, *39*(suppl_1), D561-D568. https://doi.org/10.1093/nar/gkq973

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., ... & von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic acids research*, *51*(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000

Tam, E. W., Chen, J. H., Lau, E. C., Ngan, A. H., Fung, K. S., Lee, K. C., ... & Woo, P. C. (2014). Misidentification of Aspergillus nomius and Aspergillus tamarii as Aspergillus flavus: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, *52*(4), 1153-1160. https://doi.org/10.1128/JCM.03258-13

Von Hippel, P. H., Bear, D. G., Morgan, W. D., & McSwiggen, J. A. (1984). Protein-nucleic acid interactions in transcription: a molecular analysis. *Annual Review of Biochemistry*, *53*(1), 389-446. https://doi.org/10.1146/annurev.bi.53.070184.002133

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research*, *46*(W1), W296-W303. https://doi.org/10.1093/nar/gky427

Yu, J., Cleveland, T. E., Nierman, W. C., & Bennett, J. W. (2005). Aspergillus flavus genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *Revista iberoamericana de micología*, *22*(4), 194-202. https://doi.org/10.1016/S1130-1406(05)70043-7

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi chemical society*, *15*(2), 129-144. https://doi.org/10.1016/j.jscs.2010.06.006

Second revision: Minor revisions (05-12-2023)

In Silico Analysis of *Aspergillus flavus* Fungal Proteins: Structural and Functional Insights Using ITS Primers

Mariana Mariana, Saipul Abbas*, Salamiah Salamiah, Samharinto Samharinto, Yusriadi Marsuni, Muslimin Sepe,
Muhammad Indar Pramudi, Ismed Setya Budi, Dewi Fitriyanti, Elly Liestiany, Lyswiana Aphrodyanti

Department of Plant Protection, Faculty of Agriculture, Lambung Mangkurat University. Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia

*Corresponding author: Email: saipul.abbas@ulm.ac.id

ABSTRACT

Aspergillus flavus is a type of fungus 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 Expasy 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*, *Aspergillus tamarii*, and *Aspergillus nomius*. Furthermore, the results of translating DNA sequences into proteins using Web Expasy 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

Formatted: Font: (Default) Times New Roman, Font color: Custom Color(RGB(13;13;13)), Highlight
Formatted: Font: (Default) Times New Roman, Font color: Custom Color(RGB(13;13;13))
Formatted: Font: (Default) Times New Roman
Formatted: Normal, Indent: Left: 0,63 cm, No bullets or numbering
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Line spacing: Multiple 1,15 li
Comment [SB1]: Title is not suitable according to the scope of journal and is not seem meaningful
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman
Formatted: Highlight
Comment [SB2]: Please rewrite to make it more clear so that it can impact your starting
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Highlight
Comment [SB3]: Objective of study is not elaborated well, please mention the purpose of structure and function of proteins
Formatted: Highlight
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Highlight
Comment [SB4]: You are writing same thing again and again
Formatted ...
Comment [SB5]: So?
Formatted: Highlight
Formatted ...
Formatted: Highlight
Formatted: Font: (Default) Times New Roman
Comment [SB6]: What do you want to say
Formatted: Font: (Default) Times New Roman
Formatted ...
Formatted ...
Formatted: Font: (Default) Times New Roman

processed products (Ayofemi Olalekan Adeyeye, 2020). One of ~~–~~the fungi Aspergillus spp that can contaminate feed ingredients is *the species Aspergillus flavus* which can cause aflatoxin pollution (Klich, 2007), which is a harmful substance that can affect human health and livestock (Balina et al, 2018). *Aspergillus 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 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~~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 expected to contribute to the development of methods for detecting and preventing contamination of Aspergillus spp. in corn feed and other foodstuffs.

Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman

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'-TCCGTAGGTGAACCTGCGG-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,

Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Comment [SB7]: Write identify instead of verify
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, Font color: Red
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Comment [SB8]: Please rewrite to convey right meaning
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman
Comment [SB9]: What does it means
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Comment [SB10]: I am sorry to say, but writeup is not good, please try to write in better way
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, Highlight
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman

Aspergillus 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 (Szklarczyk 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~~analysed and how the protein interaction was related to the biological function found. This method could be used to ~~analyze~~analysed 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.

Comment [SB11]: Please do cut short

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman

RESULTS AND DISCUSSION

Formatted: Font: (Default) Times New Roman

Identification of fungus species by BLAST Method

Formatted: Font: (Default) Times New Roman

The results of the fungal~~us~~ 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 *Aspergillus tamarii* compared to the other two species. *Aspergillus tamarii* is recognized for its capability to produce various bioactive compounds (Bose et al, 2019), including phenol, flavonoid and IAA and its antifungal activity, which have potential applications in the Agriculture, and food industries. However, it is important to note that *Aspergillus tamarii* can also cause infections in humans and animals (Tam et al, 2014). Similarly, *Aspergillus 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, *Aspergillus nomius*, another identified species in the sample, can produce aflatoxin and 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 *Aspergillus tamarii* and *Aspergillus 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.

Formatted: Font: (Default) Times New Roman

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

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Comment [SB12]: Please give short descriptions

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: 12 pt

Formatted: Font: 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: 12 pt

MNGGPAEATKVQ	12	Peptide	A1
MAGPPKQLRYSKHGWEVGLARNPTLGND PSVGEPAEGSLPSVGFLASPTSHPCLLYLSC FGGPAIHGRRGLSAPGPRPPETPRTLSDLV KSELIVSQSVKTFNNGSLGSGIDEERSEMR	119	Protein	A2
MPVRASLLPIKHGLCVGSSSPLRGGRAPKA AAAPRPILERMGLCHPLCRPGRRLPNANQS FSRLTSDQVGIPAELKHINKAEE	83	Protein	A3
MILP	4	Peptide	A4
MAY	4	Peptide	A5
MAAGGSQPRARARRRHHELCLI	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 show 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 it can be used as a basis for specific drug and therapeutic development.

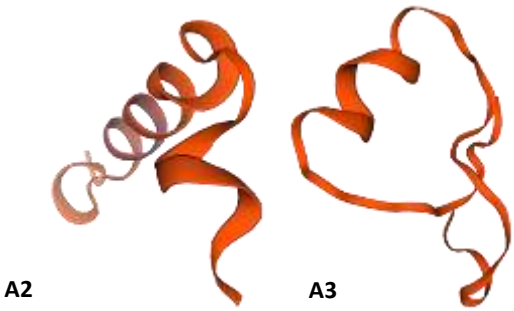


Figure 3: Protein Structure Prediction from Internal Transcribed Spacer (ITS) primer *of Aspergillus flavus* fungus using Protein Prediction from SWISS Model. (Template: A2: 3lb6.1.A, A3: 5t7v.1.K)

The results of validation of the *Aspergillus spp* protein structure using SAVES V6.0 show 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 are 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.

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~~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~~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

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman, Font color: Auto, English (Indonesia)

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Comment [SB13]: Drug development for what

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, Font color: Red

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

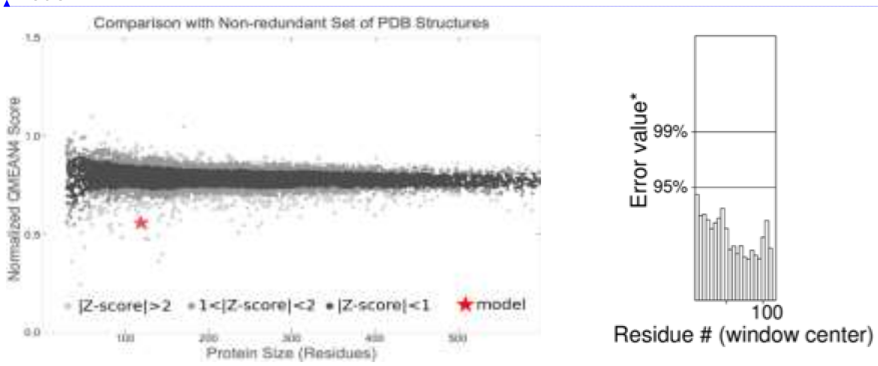
Formatted: Font: (Default) Times New Roman

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.

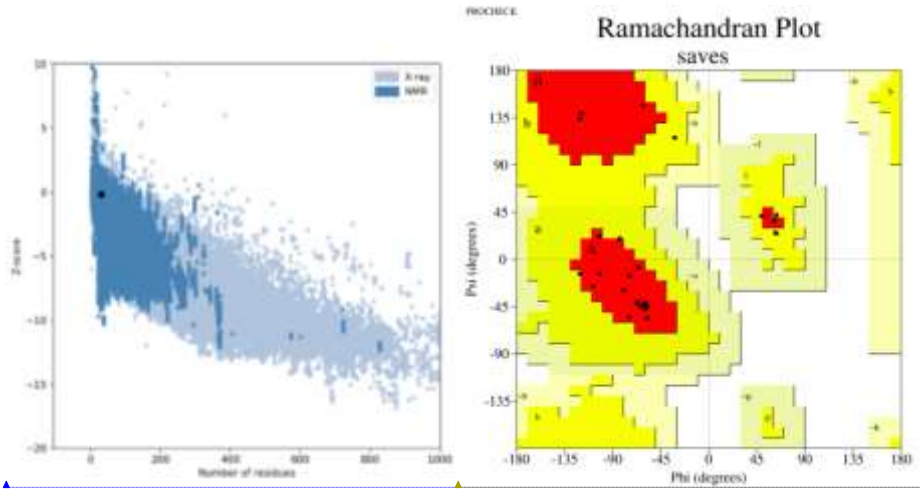
Table 2: Protein Structure Validation using SAVES V6.0

Protein Code	Ramachandran plot				ERRAT quality factor (%)	QMEAN4 ~~Score~~score	Z Score
	~~Residu~~Residue in most favored regions (%)	~~Residu~~Residue in additional allowed regions (%)	~~Residu~~Residue in generously allowed regions (%)	~~Residu~~Residue 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

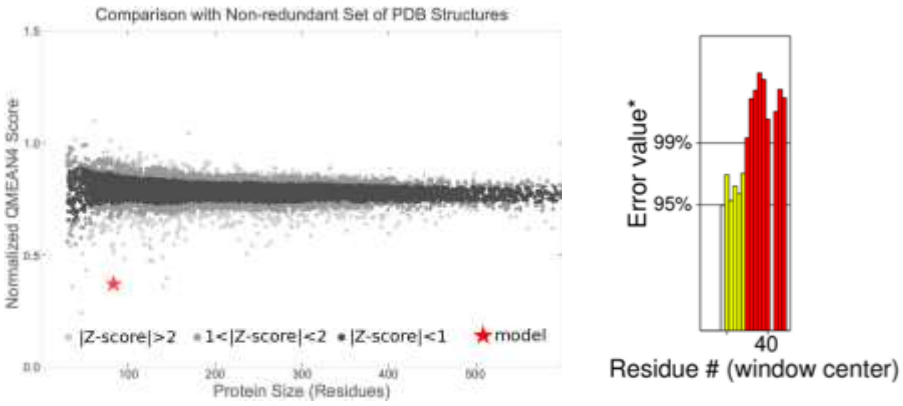
Model A2



Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Roman
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Roman



Model A3



XXX

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

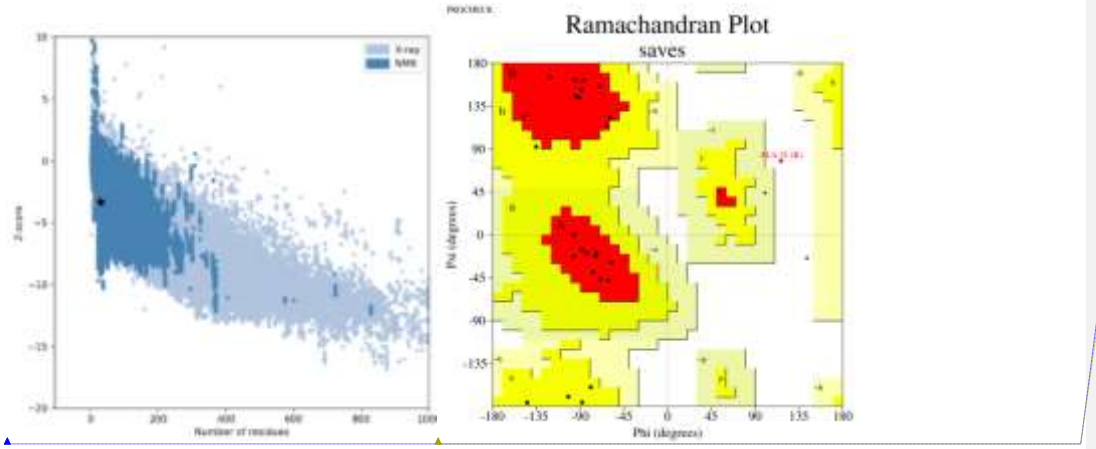


Figure 4: The results of validation of the *Aspergillus spp* protein ~~structure using~~structure using SAVES V6.0

Protein Function & Interaction of *Aspergillus Flavus*

Aspergillus 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 *Aspergillus 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 *Aspergillus flavus* survive in a poor environment (Abrar et al, 2013). (3) As an antifungal compound: Aflatoxin can help the fungus *Aspergillus flavus* resist attacks from other fungi that seek to interfere with the growth of the fungus *Aspergillus flavus* (Bhatnagar-Mathur et al, 2015). Overall, heterocyclic in aflatoxins has an important role in the biology of the fungus *Aspergillus 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 *Aspergillus flavus*. Organic chemical compounds that bind to the fungus *Aspergillus flavus* can affect the biological activity of the fungus (Nguyen et al, 2019). Some organic chemical compounds that can bind to the fungus *Aspergillus flavus* include growth regulators, metabolic regulators, and anti-fungal substances (Hou et al, 2022). The binding of organic chemical compounds to the fungus *Aspergillus*

Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman

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

Formatted: Font: (Default) Times New Roman

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, *Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus flavus* may help in understanding the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

Formatted: Font: (Default) Times New Roman

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

Formatted: Font: (Default) Times New Roman

facilitate interaction with enzymes and transcription factors (Corley et al, 2020). In the fungus Aspergillus 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 *Aspergillus 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 *Aspergillus 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 Aspergillus 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 Aspergillus 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 *Aspergillus 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 *Aspergillus 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 *Aspergillus flavus* can help in understanding the mechanisms of fungal growth and reproduction, as well as how the fungus reacts to its environment.

Formatted: Font: (Default) Times New Roman

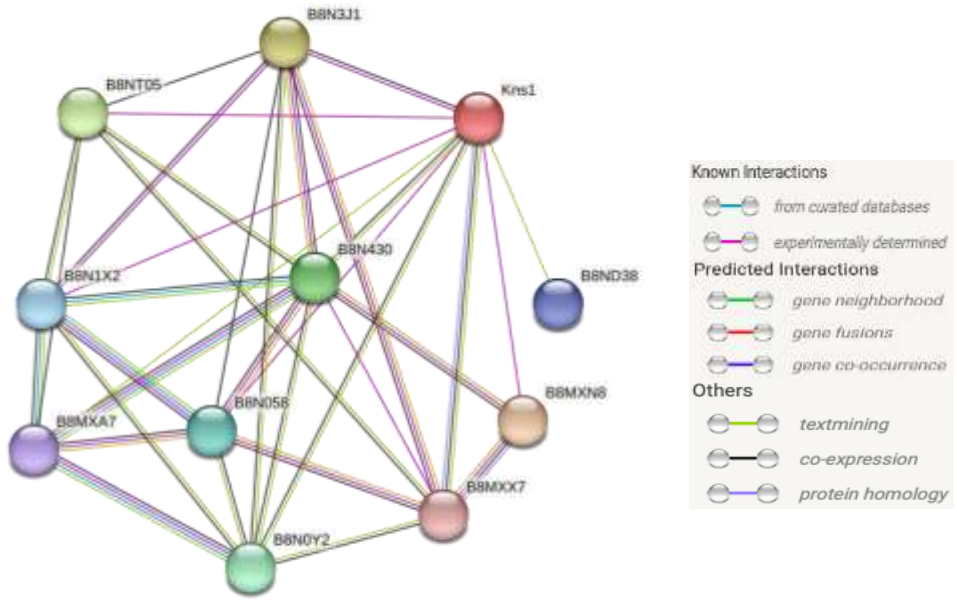


Figure 5: Network of protein-protein interactions visualized with STRING (NCBI taxon-Id: 332952)

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	Sucellular Localization
• Heterocyclic compound binding	• mRNA splicing, via spliceosome • RNA processing	• Spliceosomal complex • Ribonucleoprotein	• U2 snRNP • U2-type prespliceosome • U2-type spliceosomal

• Organic cyclic compound binding • Nucleic acid binding • RNA binding • mRNA binding • Structural constituent of ribosome • rRNA binding • snoRNA binding	• Gene expression • Macromolecule metabolic process • Cellular nitrogen compound metabolic process • Nitrogen compound metabolic process • Primary metabolic process • Cellular metabolic process	complex • Nucleus • Intracellular membrane-bounded organelle	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, *Aspergillus tamarii*, ~~and *Aspergillus*~~and Aspergillus *nomius*. Translating DNA sequences into proteins using Web Expasy 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 thatshows 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 offunction 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.

Acknowledgement

A thank you to PT. Arutmin Indonesia Tambang Satui and Agreement/Contract Number: 39/UN8.1.23/PP/2022 through the Matching Fund program "Kedaireka" for financial support for this research activity, and all parties who have helped in the success of this research. All the help and support provided means a lot to us. Thanks.

Reference

Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Line spacing: Multiple 1,15 li
Formatted: Line spacing: Multiple 1,15 li
Formatted: Line spacing: Multiple 1,15 li
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman
Formatted: Font: (Default) Times New Roman

Ayaz, A., Zaman, W., Saqib, S., Ullah, F., & Mahmood, T. (2020). Phylogeny and diversity of Lamiaceae based on rps14 gene in Pakistan. *Genetika*, *52*(2), 435-452. https://doi.org/10.2298/GENSR2002435A

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). From DNA to RNA. In *Molecular Biology of the Cell. 4th edition*. Garland Science.

Abrar, M., Anjum, F. M., Butt, M. S., Pasha, I., Randhawa, M. A., Saeed, F., & Waqas, K. (2013). Aflatoxins: biosynthesis, occurrence, toxicity, and remedies. *Critical reviews in food science and nutrition*, *53*(8), 862-874. https://doi.org/10.1080/10408398.2011.563154

Ayofemi Olalekan Adeyeye, S. (2020). Aflatoxigenic fungi and mycotoxins in food: a review. *Critical reviews in food science and nutrition*, *60*(5), 709-721. https://doi.org/10.1080/10408398.2018.1548429

Amaike, S., & Keller, N. P. (2011). Aspergillus flavus. *Annual review of phytopathology*, *49*, 107-133. https://doi.org/10.1146/annurev-phyto-072910-095221

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating Aspergillus flavus and aflatoxin contamination in crops. *Plant science*, *234*, 119-132. https://doi.org/10.1016/j.plantsci.2015.02.009

Bhattacharya, A., Tejero, R., & Montelione, G. T. (2007). Evaluating protein structures determined by structural genomics consortia. *Proteins: Structure, Function, and Bioinformatics*, *66*(4), 778-795. https://doi.org/10.1002/prot.21165

Blackburn, G. M. (Ed.). (2006). *Nucleic acids in chemistry and biology*. Royal Society of Chemistry.

Breda, A., Valadares, N. F., de Souza, O. N., & Garratt, R. C. (2007). Protein structure, modelling and applications. In *Bioinformatics in tropical disease research: a practical and case-study approach [Internet]*. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK6824/

Benkert, P., Tosatto, S. C., & Schomburg, D. (2008). QMEAN: A comprehensive scoring function for model quality assessment. *Proteins: Structure, Function, and Bioinformatics*, *71*(1), 261-277. https://doi.org/10.1002/prot.21715

Benkert, P., Biasini, M., & Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, *27*(3), 343-350. https://doi.org/10.1093/bioinformatics/btq662

Bordoli, L., & Schwede, T. (2012). Automated protein structure modeling with SWISS-MODEL Workspace and the Protein Model Portal. *Homology Modeling: Methods and Protocols*, 107-136. https://doi.org/10.1007/978-1-61779-588-6_5

Bose, P., Gowrie, S. U., & Chathurdevi, G. (2019). Optimization of Culture Conditions for Growth and Production of Bioactive Metabolites by Endophytic Fungus-Aspergillus tamarii. *International Journal of Pharmacy and Biological Sciences*, *9*(2), 469-478. https://doi.org/10.21276/ijpbs.2019.9.2.60

Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, *6*(2), e555685. https://doi.org/10.19080/JDVS.2018.06.555685

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, No underline

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Bashir, S., Janiad, S., Zeeshan, A., Farah, N., Mehboob, S., Butt, H. I., & Qari, S. H. (2022). In Silico Analysis and Function Prediction of Sr22 Gene Product as Stem Rust Resistant Protein. *Annals of the Romanian Society for Cell Biology*, *26*(01), 258-262. http://annalsofrscb.ro

Cram, D. J., & Cram, J. M. (1971). Cyclophane chemistry: bent and battered benzene rings. *Accounts of Chemical Research*, *4*(6), 204-213. https://doi.org/10.1021/ar50042a003

Corley, M., Burns, M. C., & Yeo, G. W. (2020). How RNA-binding proteins interact with RNA: molecules and mechanisms. *Molecular cell*, *78*(1), 9-29. https://doi.org/10.1016/j.molcel.2020.03.011

de la Cruz, J., Karbstein, K., & Woolford Jr, J. L. (2015). Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo. *Annual review of biochemistry*, *84*, 93-129. https://doi.org/10.1146/annurev-biochem-060614-033917

Dhanamjayulu, P., Boga, R. B., & Mehta, A. (2019). Inhibition of aflatoxin B1 biosynthesis and down regulation of aflR and aflB genes in presence of benzimidazole derivatives without impairing the growth of Aspergillus flavus. *Toxicon*, *170*, 60-67. https://doi.org/10.1016/j.toxicon.2019.09.018

Damodaran, S., & Parkin, K. L. (2017). Amino acids, peptides, and proteins. In *Fennema's food chemistry* (pp. 235-356). CRC Press.

Dreyfuss, G., Kim, V. N., & Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages they carry. *Nature reviews Molecular cell biology*, *3*(3), 195-205. https://doi.org/10.1038/nrm760

Dutta, B., Banerjee, A., Chakraborty, P., & Bandopadhyay, R. (2018). In silico studies on bacterial xylanase enzyme: Structural and functional insight. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 749-756. https://doi.org/10.1016/j.jgeb.2018.05.003

Elliott, D., & Ladomery, M. (2017). *Molecular biology of RNA*. Oxford University Press.

Frank, J., & Spahn, C. M. (2006). The ribosome and the mechanism of protein synthesis. *Reports on Progress in Physics*, *69*(5), 1383. https://doi.org/10.1088/0034-4885/69/5/R03

Fouad, A. M., Ruan, D., El-Senousey, H. K., Chen, W., Jiang, S., & Zheng, C. (2019). Harmful effects and control strategies of aflatoxin b1 produced by Aspergillus flavus and Aspergillus parasiticus strains on poultry. *Toxins*, *11*(3), 176. https://doi.org/10.3390/toxins11030176

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., ... & Jensen, L. J. (2012). STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic acids research*, *41*(D1), D808-D815. https://doi.org/10.1093/nar/gks1094

Glisovic, T., Bachorik, J. L., Yong, J., & Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. *FEBS letters*, *582*(14), 1977-1986. https://doi.org/10.1016/j.febslet.2008.03.004

Gourama, H., & Bullerman, L. B. (1995). Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food protection*, *58*(12), 1395-1404. https://doi.org/10.4315/0362-028X-58.12.1395

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic acids research*, *31*(13), 3784-3788. https://doi.org/10.1093/nar/gkg563

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, *47*, 101716. https://doi.org/10.1016/j.fbio.2022.101716

Kirubakaran, P., Karthikeyan, M., Singh, K. D., Nagamani, S., & Premkumar, K. (2013). In silico structural and functional analysis of the human TOPK protein by structure modeling and molecular dynamics studies. *Journal of molecular modeling*, *19*, 407-419. https://doi.org/10.1007/s00894-012-1566-1

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, *8*(6), 713-722. https://doi.org/10.1111/j.1364-3703.2007.00436.x

Laskowski, R. A., Furnham, N., & Thornton, J. M. (2013). The Ramachandran plot and protein structure validation. In *Biomolecular forms and functions: a celebration of 50 years of the ramachandran map* (pp. 62-75). https://doi.org/10.1142/9789814449144_0005

Lake, J. A. (1981). The ribosome. *Scientific American*, *245*(2), 84-97. https://doi.org/10.1038/scientificamerican0881-84

Lovell, S. C., Davis, I. W., Arendall III, W. B., De Bakker, P. I., Word, J. M., Prisant, M. G., ... & Richardson, D. C. (2003). Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics*, *50*(3), 437-450. https://doi.org/10.1002/prot.10286

Lobo, I. (2008). Basic local alignment search tool (BLAST). *Nature Education*, *1*(1).

Nguyen, H. N., Chaves-Lopez, C., Oliveira, R. C., Paparella, A., & Rodrigues, D. F. (2019). Cellular and metabolic approaches to investigate the effects of graphene and graphene oxide in the fungi Aspergillus flavus and Aspergillus niger. *Carbon*, *143*, 419-429. https://doi.org/10.1016/j.carbon.2018.10.099

Ogodo, A. C., & Ugbogu, O. C. (2016). Public health significance of aflatoxin in food industry—A review. *European Journal of Clinical and Biomedical Sciences*, *2*(5), 51-58. https://doi.org/10.11648/j.ejcbs.20160205.14

Rozario, L. T., Sharker, T., & Nila, T. A. (2021). In silico analysis of deleterious SNPs of human MTUS1 gene and their impacts on subsequent protein structure and function. *PloS one*, *16*(6), e0252932. https://doi.org/10.1371/journal.pone.0252932

Ramirez, M. L., Cendoya, E., Nichea, M. J., Zachetti, V. G. L., & Chulze, S. N. (2018). Impact of toxigenic fungi and mycotoxins in chickpea: A review. *Current Opinion in Food Science*, *23*, 32-37. https://doi.org/10.1016/j.cofs.2018.05.003

Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., ... & Mering, C. V. (2010). The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic acids research*, *39*(suppl_1), D561-D568. https://doi.org/10.1093/nar/gkq973

Formatted: Default Paragraph Font, Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., ... & von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic acids research*, *51*(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000

Tam, E. W., Chen, J. H., Lau, E. C., Ngan, A. H., Fung, K. S., Lee, K. C., ... & Woo, P. C. (2014). Misidentification of Aspergillus nomius and Aspergillus tamarii as Aspergillus flavus: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, *52*(4), 1153-1160. https://doi.org/10.1128/JCM.03258-13

Von Hippel, P. H., Bear, D. G., Morgan, W. D., & McSwiggen, J. A. (1984). Protein-nucleic acid interactions in transcription: a molecular analysis. *Annual Review of Biochemistry*, *53*(1), 389-446. https://doi.org/10.1146/annurev.bi.53.070184.002133

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research*, *46*(W1), W296-W303. https://doi.org/10.1093/nar/gky427

Yu, J., Cleveland, T. E., Nierman, W. C., & Bennett, J. W. (2005). Aspergillus flavus genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *Revista iberoamericana de micología*, *22*(4), 194-202. https://doi.org/10.1016/S1130-1406(05)70043-7

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi chemical society*, *15*(2), 129-144. https://doi.org/10.1016/j.jscs.2010.06.006

Copyeditting Revision (27-12-2023)

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

http://www.pakps.com

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

Mariana Mariana, Saipul Abbas*, Salamiah Salamiah, Samharinto Samharinto, Yusriadi Marsuni, Muslimin Sepe,
Muhammad I. Pramudi, Ismed S. Budi, Dewi Fitriyanti, Elly Liestiany, Lyswiana Aphrodyanti

Department of Plant Protection, Faculty of Agriculture, Lambung Mangkurat University. Jl.Jend. A. Yani Km.36 Banjarbaru. South Kalimantan 70714 Indonesia.

ABSTRACT

XXX

Formatted: Highlight
Formatted: Highlight
Formatted: Font: (Default) Times New Roman
Formatted: Condensed by 0,2 pt

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 Expasy 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 Expasy 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 products, especially in food crops, rice, and legumes (Amaike & Keller, 2011). Contamination by *Aspergillus* spp. can 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*

Submitted: June 28, 2023
Revised: July 17, 2023
Accepted for Publication: December 25, 2021
*Corresponding Author:
Email: saipul.abbas@ulm.ac.id

Formatted: Expanded by 0,15 pt

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'-TCCGTAGGTGAACCTGCGG-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.

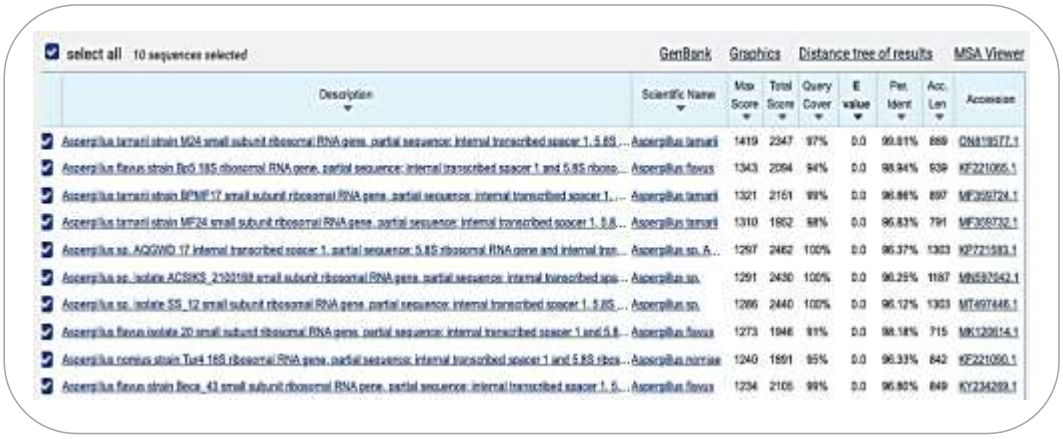
Analysis of protein structure: This stage used the SWISS Model method to predict the protein structure of the fungus *Aspergillus* spp. At this stage, the input of protein sequencing data from Expasy tools was carried out, and then protein modeling was made using the SWISS Model (Waterhouse et al, 2018). 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 (Szklarczyk 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 the 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 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
Aspergillus tamarii strain M24 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Aspergillus tamarii	1419	2347	97%	0.0	99.81%	869	ON819577.1
Aspergillus flavus strain Bp5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosp...	Aspergillus flavus	1343	2094	94%	0.0	98.94%	939	KF221065.1
Aspergillus tamarii strain BPMF17 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Aspergillus tamarii	1321	2151	99%	0.0	96.86%	897	MF359724.1
Aspergillus tamarii strain MF24 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Aspergillus tamarii	1310	1862	98%	0.0	96.83%	791	MF359732.1
Aspergillus sp. AQGWD 17 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran...	Aspergillus sp. A...	1297	2462	100%	0.0	96.37%	1303	KP721593.1
Aspergillus sp. isolate ACSIKS_2120198 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Aspergillus sp.	1291	2430	100%	0.0	96.25%	1187	MN597042.1
Aspergillus sp. isolate SS_12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Aspergillus sp.	1286	2440	100%	0.0	96.12%	1303	MT497446.1
Aspergillus flavus isolate 20 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8...	Aspergillus flavus	1273	1946	91%	0.0	98.18%	715	MK120614.1
Aspergillus nomius strain Tur4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribos...	Aspergillus nomius	1240	1891	95%	0.0	96.33%	842	KF221050.1
Aspergillus flavus strain Ilboa_43 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	Aspergillus flavus	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.

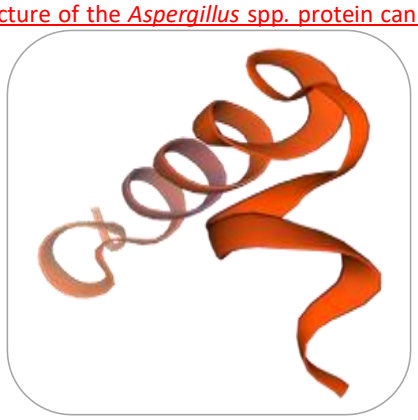
Figure 2. The results of *Aspergillus* spp. DNA sequences into proteins using the ExPASy web

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
MAGPPKQLRYSKHGWEVGLARNPTLGNDPSVGEPAEGSLPSVGFLASPTSHPCLLYLSCFGGPAIHGRRGLSAPGPRPPETPRTLSDLVKSELIVSQSVKTFNNGSLGSGIDEERSEMR	119	Protein	A2
MPVRASLLPIKHGLCVGSSSPLRGGRAPKAAAPRPILERMGLCHPLCRPGRRLPNANQSFSRLTSDQVGIPAELKHINKAEE	83	Protein	A3
MILP	4	Peptide	A4
MAY	4	Peptide	A5
MAAGGSQPRARARRRHHELCLI	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 show 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 it can be used as a basis for specific drug and therapeutic development.



A2

A3

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 show 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 are 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.

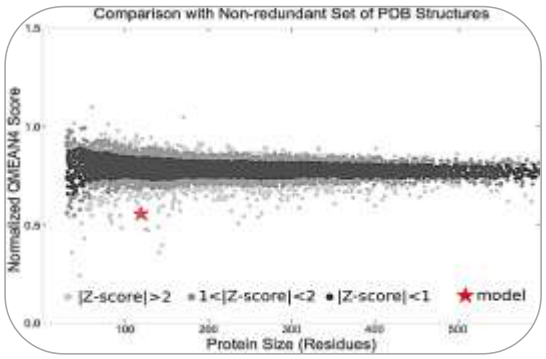
The QMEAN4 Score indicates the overall quality of the protein structure, which is calculated based on geometric values, non-covalent interactions, and consistency of the structure (Benkert et al, 2008). The higher the QMEAN 4 Score, the better the quality of the resulting protein structure (Benkert et 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

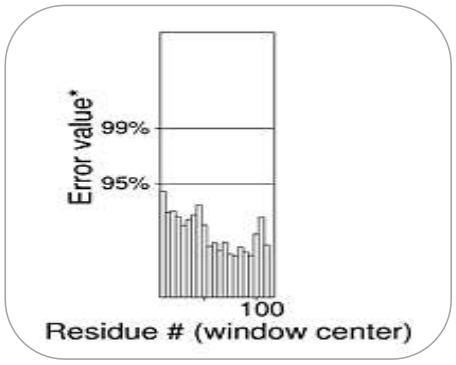
geometric values and structural consistency. The higher the Z Score value, the better the quality of the resulting protein structure compared to other similar protein structures (Bhattacharya et al, 2007). In this case, a Z Score of -0.16 indicates that the resulting *Aspergillus* spp. protein structure 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.

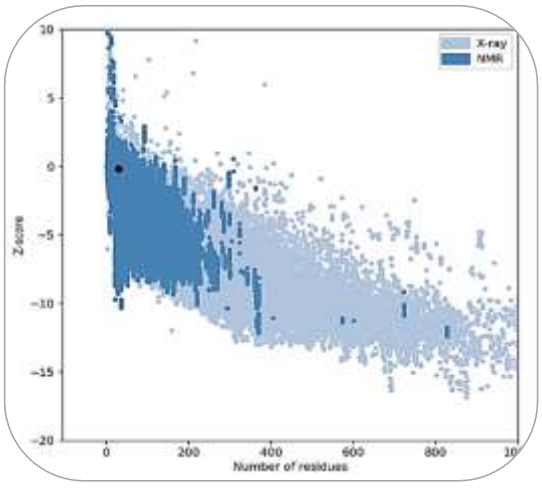
Table 2. Protein Structure Validation using SAVES V6.0

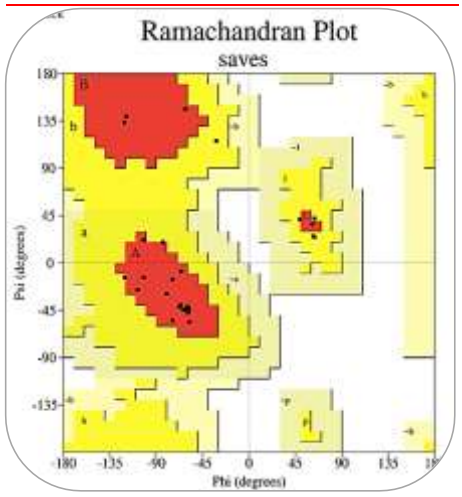
Protein Code	Ramachandran plot				ERRAT quality factor (%)	QMEAN 4 Score	Z Score
	Residu in most favored regions (%)	Residu in additional allowed regions (%)	Residu in generously allowed regions (%)	Residu in disallowed regions (%)			
A2	80.0	20.0	0.0	0.0	100	-1.72	-0.16
A3	75.0	20.0	0.0	5.0	6.6666	-3.27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

Model A2









Model A3

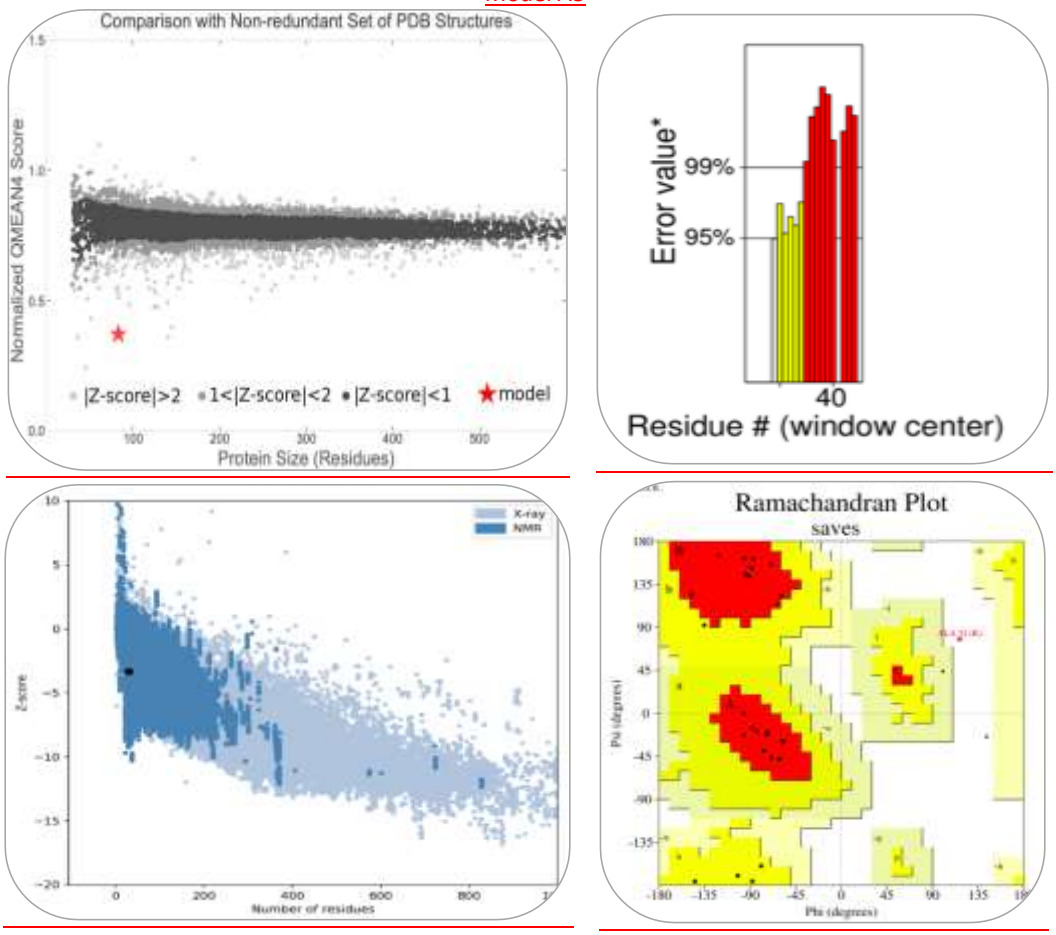


Figure 4. The results of validation of the *Aspergillus* spp. protein structure using SAVES V6.0

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.

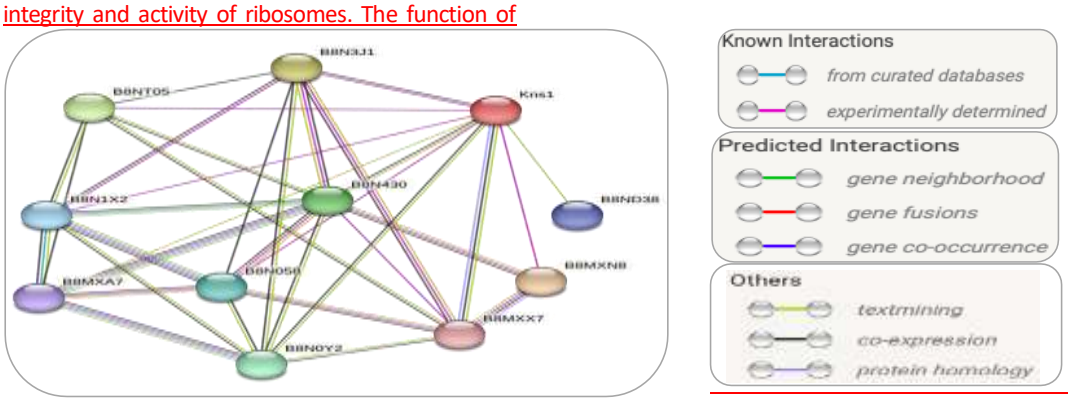
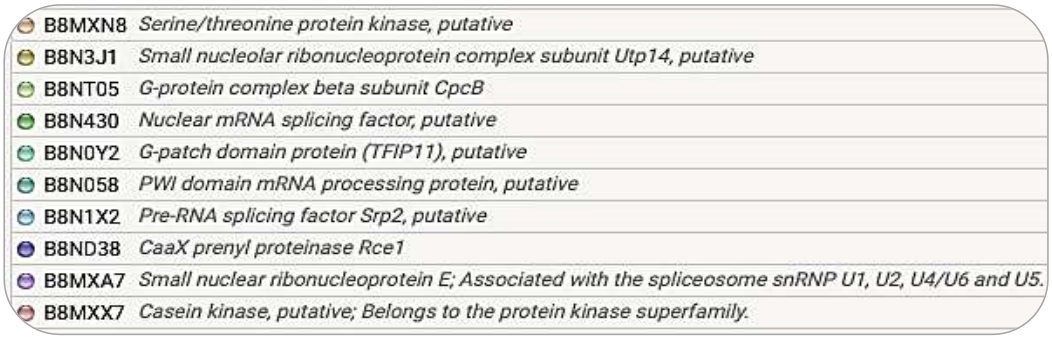


Figure 5: Network of protein-protein interactions visualized with STRING (NCBI taxon-Id: 332952)



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	Sucellular Localization
• Heterocyclic compound binding • Organic cyclic compound binding • Nucleic acid binding • RNA binding • mRNA binding • Structural constituent of ribosome • rRNA binding • snoRNA binding	• 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	• Spliceosomal complex • Ribonucleoprotein complex • Nucleus • Intracellular membrane-bounded organelle	• 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 Expasy 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.

ACKNOWLEDGEMENT

A thank you to PT. Arutmin Indonesia Tambang Satui and Agreement/Contract Number: 39/UN8.1.23/PP/2022 through the Matching Fund program "Kedaireka" for financial support for this research activity, and all parties who have helped in the success of this research. All the help and support provided means a lot to us. Thanks.

REFERENCES

Ayaz, A., Zaman, W., Saqib, S., Ullah, F., & Mahmood, T. (2020). Phylogeny and diversity of Lamiaceae based on rps14 gene in Pakistan. *Genetika*, *52*(2), 435-452. https://doi.org/10.2298/GENSR2002435A

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts,

K., & Walter, P. (2002). From DNA to RNA. In *Molecular Biology of the Cell. 4th edition*. Garland Science.

Abrar, M., Anjum, F. M., Butt, M. S., Pasha, I., Randhawa, M. A., Saeed, F., & Waqas, K. (2013). Aflatoxins: biosynthesis, occurrence, toxicity, and remedies. *Critical reviews in food science and nutrition*, *53*(8), 862-874. https://doi.org/10.1080/10408398.2011.563154

Ayofemi Olalekan Adeyeye, S. (2020). Aflatoxigenic fungi and mycotoxins in food: a review. *Critical reviews in food science and nutrition*, *60*(5), 709-721. https://doi.org/10.1080/10408398.2018.1548429

Amaike, S., & Keller, N. P. (2011). Aspergillus flavus. *Annual review of phytopathology*, *49*, 107-133. https://doi.org/10.1146/annurev-phyto-072910-095221

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating Aspergillus flavus and aflatoxin contamination in crops. *Plant science*, *234*, 119-132. https://doi.org/10.1016/j.plantsci.2015.02.009

Bhattacharya, A., Tejero, R., & Montelione, G. T. (2007). Evaluating protein structures determined by structural genomics consortia. *Proteins: Structure, Function, and Bioinformatics*, *66*(4), 778-795. https://doi.org/10.1002/prot.21165

Blackburn, G. M. (Ed.). (2006). *Nucleic acids in chemistry and biology*. Royal Society of Chemistry.

Breda, A., Valadares, N. F., de Souza, O. N., & Garratt, R. C. (2007). Protein structure, modelling and applications. In *Bioinformatics in tropical disease research: a practical and case-study approach [Internet]*. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK6824/

Benkert, P., Tosatto, S. C., & Schomburg, D. (2008). QMEAN: A comprehensive scoring function for model quality assessment. *Proteins: Structure, Function, and Bioinformatics*, *71*(1), 261-277. https://doi.org/10.1002/prot.21715

Benkert, P., Biasini, M., & Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, *27*(3), 343-350. https://doi.org/10.1093/bioinformatics/btq662

Bordoli, L., & Schwede, T. (2012). Automated protein structure modeling with SWISS-MODEL Workspace and the Protein Model Portal. *Homology Modeling: Methods and Protocols*, 107-136. https://doi.org/10.1007/978-1-61779-588-6_5

Bose, P., Gowrie, S. U., & Chathurdevi, G. (2019). Optimization of Culture Conditions for Growth and Production of Bioactive Metabolites by Endophytic Fungus-Aspergillus tamarii. *International Journal of Pharmacy and Biological Sciences*, *9*(2), 469-478. https://doi.org/10.21276/ijpbs.2019.9.2.60

Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, *6*(2), e555685. https://doi.org/10.19080/JDVS.2018.06.555685

Bashir, S., Janiad, S., Zeeshan, A., Farah, N., Mehboob, S., Butt, H. I., & Qari, S. H. (2022). In Silico Analysis and Function Prediction of Sr22 Gene Product as Stem Rust Resistant Protein. *Annals of the Romanian Society for Cell Biology*, *26*(01), 258-262. http://annalsofrscb.ro

Cram, D. J., & Cram, J. M. (1971). Cyclophane chemistry: bent and battered benzene rings. *Accounts of Chemical Research*, *4*(6), 204-

213. https://doi.org/10.1021/ar50042a003

Corley, M., Burns, M. C., & Yeo, G. W. (2020). How RNA-binding proteins interact with RNA: molecules and mechanisms. *Molecular cell*, *78*(1), 9-29. https://doi.org/10.1016/j.molcel.2020.03.011

de la Cruz, J., Karbstein, K., & Woolford Jr, J. L. (2015). Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo. *Annual review of biochemistry*, *84*, 93-129. https://doi.org/10.1146/annurev-biochem-060614-033917

Dhanamjayulu, P., Boga, R. B., & Mehta, A. (2019). Inhibition of aflatoxin B1 biosynthesis and down regulation of aflR and aflB genes in presence of benzimidazole derivatives without impairing the growth of Aspergillus flavus. *Toxicon*, *170*, 60-67. https://doi.org/10.1016/j.toxicon.2019.09.018

Damodaran, S., & Parkin, K. L. (2017). Amino acids, peptides, and proteins. In *Fennema's food chemistry* (pp. 235-356). CRC Press.

Dreyfuss, G., Kim, V. N., & Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages they carry. *Nature reviews Molecular cell biology*, *3*(3), 195-205. https://doi.org/10.1038/nrm760

Dutta, B., Banerjee, A., Chakraborty, P., & Bandopadhyay, R. (2018). In silico studies on bacterial xylanase enzyme: Structural and functional insight. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 749-756. https://doi.org/10.1016/j.jgeb.2018.05.003

Elliott, D., & Ladomery, M. (2017). *Molecular biology of RNA*. Oxford University Press.

Frank, J., & Spahn, C. M. (2006). The ribosome and the mechanism of protein synthesis. *Reports on Progress in Physics*, *69*(5), 1383. https://doi.org/10.1088/0034-4885/69/5/R03

Fouad, A. M., Ruan, D., El-Senousey, H. K., Chen, W., Jiang, S., & Zheng, C. (2019). Harmful effects and control strategies of aflatoxin b1 produced by Aspergillus flavus and Aspergillus parasiticus strains on poultry. *Toxins*, *11*(3), 176. https://doi.org/10.3390/toxins11030176

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., ... & Jensen, L. J. (2012). STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic acids research*, *41*(D1), D808-D815. https://doi.org/10.1093/nar/gks1094

Glisovic, T., Bachorik, J. L., Yong, J., & Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. *FEBS letters*, *582*(14), 1977-1986. https://doi.org/10.1016/j.febslet.2008.03.004

Gourama, H., & Bullerman, L. B. (1995). Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food protection*, *58*(12), 1395-1404. https://doi.org/10.4315/0362-028X-58.12.1395

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic acids research*, *31*(13), 3784-3788. https://doi.org/10.1093/nar/gkg563

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, *47*, 101716. https://doi.org/10.1016/j.fbio.2022.101716

Kirubakaran, P., Karthikeyan, M., Singh, K. D., Nagamani, S., & Premkumar, K. (2013). In silico structural and functional analysis of the human TOPK protein by structure modeling and molecular dynamics studies. *Journal of molecular modeling*, *19*, 407-419.

https://doi.org/10.1007/s00894-012-1566-1

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, *8*(6), 713-722. https://doi.org/10.1111/j.1364-3703.2007.00436.x

Laskowski, R. A., Furnham, N., & Thornton, J. M. (2013). The Ramachandran plot and protein structure validation. In *Biomolecular forms and functions: a celebration of 50 years of the ramachandran map* (pp. 62-75). https://doi.org/10.1142/9789814449144_0005

Lake, J. A. (1981). The ribosome. *Scientific American*, *245*(2), 84-97. https://doi.org/10.1038/scientificamerican0881-84

Lovell, S. C., Davis, I. W., Arendall III, W. B., De Bakker, P. I., Word, J. M., Prisant, M. G., ... & Richardson, D. C. (2003). Structure validation by Cα geometry: φ, ψ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics*, *50*(3), 437-450. https://doi.org/10.1002/prot.10286

Lobo, I. (2008). Basic local alignment search tool (BLAST). *Nature Education*, *1*(1).

Nguyen, H. N., Chaves-Lopez, C., Oliveira, R. C., Paparella, A., & Rodrigues, D. F. (2019). Cellular and metabolic approaches to investigate the effects of graphene and graphene oxide in the fungi Aspergillus flavus and Aspergillus niger. *Carbon*, *143*, 419-429. https://doi.org/10.1016/j.carbon.2018.10.099

Ogodo, A. C., & Ugbogu, O. C. (2016). Public health significance of aflatoxin in food industry—A review. *European Journal of Clinical and Biomedical Sciences*, *2*(5), 51-58. https://doi.org/10.11648/j.ejcbs.20160205.14

Rozario, L. T., Sharker, T., & Nila, T. A. (2021). In silico analysis of deleterious SNPs of human MTUS1 gene and their impacts on subsequent protein structure and function. *PloS one*, *16*(6), e0252932. https://doi.org/10.1371/journal.pone.0252932

Ramirez, M. L., Cendoya, E., Nichea, M. J., Zachetti, V. G. L., & Chulze, S. N. (2018). Impact of toxigenic fungi and mycotoxins in chickpea: A review. *Current Opinion in Food Science*, *23*, 32-37. https://doi.org/10.1016/j.cofs.2018.05.003

Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., & Mering, C. V. (2010). The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic acids research*, *39*(suppl_1), D561-D568. https://doi.org/10.1093/nar/gkq973

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., ... & von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic acids research*, *51*(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000

Tam, E. W., Chen, J. H., Lau, E. C., Ngan, A. H., Fung, K. S., Lee, K. C., & Woo, P. C. (2014). Misidentification of Aspergillus nomius and Aspergillus tamarii as Aspergillus flavus: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, *52*(4), 1153-1160. https://doi.org/ 10.1128/JCM.03258-13

Von Hippel, P. H., Bear, D. G., Morgan, W. D., & McSwiggen, J. A. (1984). Protein-nucleic acid interactions in transcription: a molecular analysis. *Annual Review of Biochemistry*, *53*(1), 389-446. https://doi.org/10.1146/annurev.bi.53.070184.002133

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... &

Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research*, *46*(W1), W296-W303. https://doi.org/10.1093/nar/gky427

Yu, J., Cleveland, T. E., Nierman, W. C., & Bennett, J. W. (2005). Aspergillus flavus genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *Revista iberoamericana de micología*, *22*(4), 194-202. https://doi.org/10.1016/S1130-1406(05)70043-7

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi chemical society*, *15*(2), 129-144. https://doi.org/10.1016/j.jscs.2010.06.006

Contribution of Authors:

Mariana Mariana	:
Saipul Abbas	:
Salamiah Salamiah	:
Samharinto Samharinto	:
Yusriadi Marsuni	:
Muslimin Sepe	:
Muhammad I. Pramudi	:
Ismed S. Budi	:
Dewi Fitriyanti	:
Elly Liestiany	:
Lyswiana Aphrodyanti	:

Formatted: Font: (Default) Times New Roma

Formatted: Line spacing: Multiple 1,15 li