Mushroom Science IXX

Science and Cultivation Of Edible Fungi

Edited by J.J.P. Baars & A.S.M. Sonnenberg

PROCEEDINGS OF THE IXXTH INTERNATIONAL CONGRESS ON THE SCIENCE AND CULTIVATION OF EDIBLE AND MEDICINAL FUNGI/AMSTERDAM/THE NETHERLANDS/30 MAY-2 JUNE 2016

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ISBN 978-90-9029771-2

How to cite this publication:

Baars J.J.P. & Sonnenberg A.S.M., ed. 2016. Science and cultivation of edible and medicinal fungi: Mushroom Science IXX. Proceedings of the 19th Congress of the International Society for Mushroom Science, Amsterdam, The Netherlands, 30 May–2 June 2016. International Society for Mushroom Science. Amsterdam

Example of how to cite a paper in this publication:

Semon S. (2016) Development of a Revised UPOV Guideline for New Agaricus Mushroom Varieties. in 'Science and cultivation of edible and medicinal fungi: Mushroom Science IXX', ed. by A.S.M. Sonnenberg & J.J.P. Baars. Proceedings of the 19th Congress of the International Society for Mushroom Science, Amsterdam, the Netherlands, 30 May–2 June 2016. International Society for Mushroom Science. Amsterdam.

Fungal Immunomodulatory Protein from Tiger Milk Mushroom, *Lignosus rhinocerotis*

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INTRODUCTION

Fungal immunomodulatory proteins (FIPs) are a family of novel proteins purified from edible medicinal mushrooms that possess extensive biological functions including anti-tumor, anti-allergy and immunomodulatory responses. To date FIP from *Lignosus rhinocerotis* (Tiger Milk Mushroom, TMM) has not yet been reported. A new FIP from TMM (FIP-Lrh) was isolated in a previous study and the protein sequence was provided. An *in silico* study was carried out to analyse the protein and compare it structurally to other FIP proteins. *L. rhinocerotis* is an important medicinal mushroom found in Malaysia and it has also been used as a food source. The sclerotium has the most medicinal value and is widely used indigenously as treatment for breast cancer, fever, cough, asthma and food poisoning among others. Biocomputational studies offer powerful new methods to predict molecular structures and understand the complex behavior of living organisms, as well as to analyze vast amount of molecular data to make predictions that guide experimental work. Homology modeling is an important technique to obtain three dimensional (3D) structure of the proteins that have not yet been identified. The goals of molecular docking are the identification of a ligand that binds to a specific receptor binding site and of its preferred, energetically most favorable, binding pose.

OBJECTIVE

•To predict the three dimensional structure of fungal immunomodulatory protein from *L. rhinocerotis* (FIP-Lrh)

•To determine the active site and important residues of the FIP-Lrh by performing docking studies on the binding site using various test ligands

METHODOLOGY

BLAST analysis of FIP-Lrh protein sequence

↓

The 3D structure of protein with nearest match (1OSY.pdb, FIP-fve template) was downloaded from PDB

↓

Modeller 9v12 was used to model the sequence

↓

Target sequence written as PIR format

".ali" file used as input for construction of 3D model	Structure with lowest objective function was selected and superimposed on template

↓

The target protein was validated using PROCHECK

↓

14 different glycans downloaded from PubChem

↓

AUTODOCK 4.2 and AUTODOCK Vina used for docking followed by analysis of glycans to both target and template protein

RESULTS

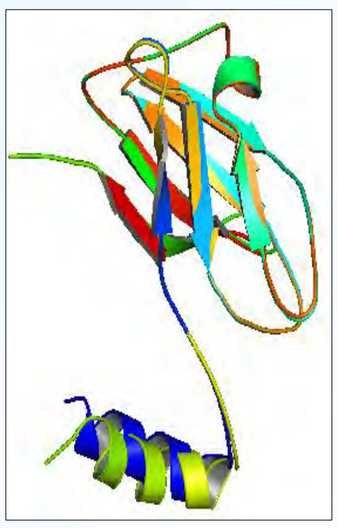

Figure 1. FIP-Lrh superimposed on the FIP-fve template protein

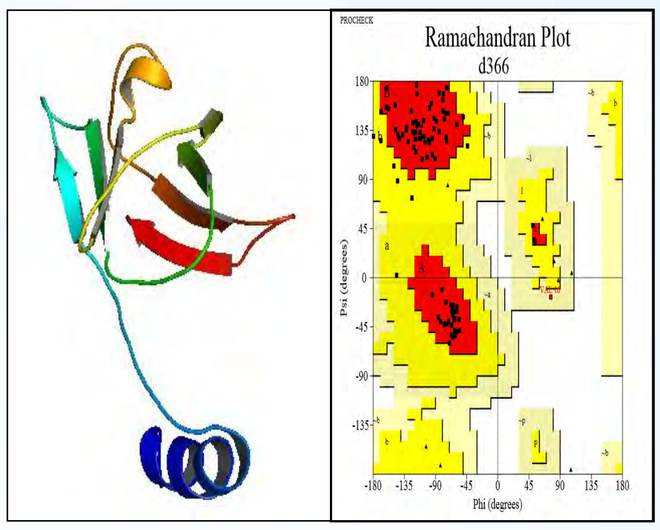

Figure 2. The Model of FIP-Lrh and its Ramachandran Plot

- The FIP-Lrh is structurally similar to FIP-fve (Fig.1).
- A total of 91.8% of the amino acids were in the core region
- Quality of the predicted structure was good (Fig. 2).

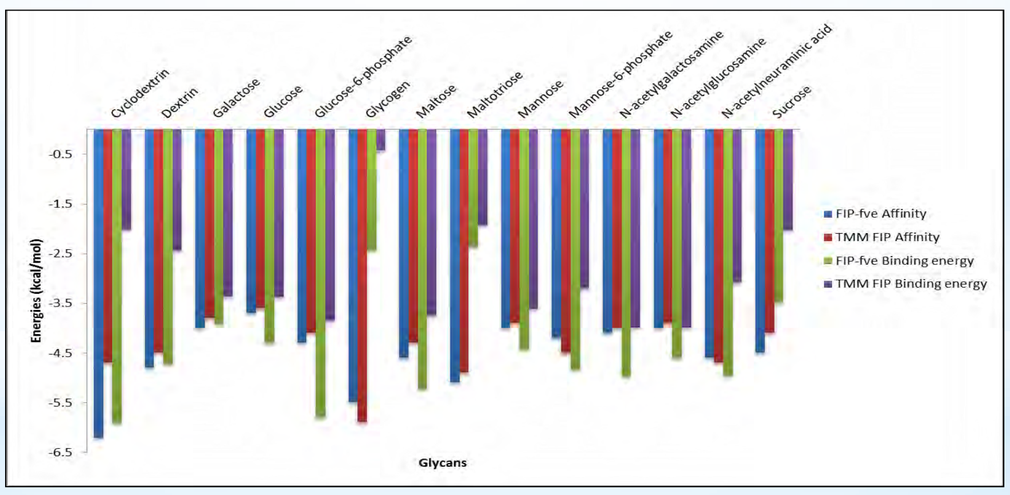

Figure 3. Chart showing the binding affinities and binding energies of the ligands

- Of a total of 14 glycans tested, glycogen had the highest binding affinity (-5.9 kcal/mol) and N-acetylgalactosamine had the highest binding energy (-3.98 kcal/mol) for the predicted protein.
- N-acetylgalactosamine docking into the binding pocket of FIP from TMM is as shown in Fig. 4.

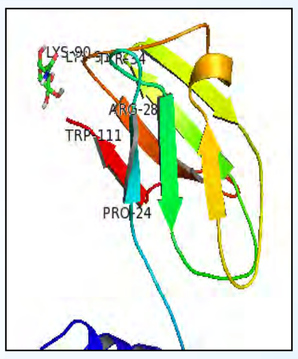

Figure 4. N-acetyl galactosamine bound to the predicted protein

CONCLUSION

- The FIP isolated form *L. rhinocerotis* was successfully modeled using FIP-fve as a template.
- Its protein sequence is similar to other FIPs and is structurally similar to FIP-fve.
- Based on the docking studies, FIP-Lrh could bind to sugars with high affinity to glycogens and N-acetylgalactosamine.
- Therefore it is predicted to properties similar to other FIPs which are lectin mitogens such as:
  - a dose dependant mitogenic effect *in vitro* for human peripheral blood lymphocytes and mouse splenocytes
  - haemagglutination
- Wet laboratory research will help to verify the results of the predictions reported in this study.

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