

FORMAMIDASE - UNDERSTANDING THE PROTEIN INTERACTION OF THE HIDDEN ENZYME IN THE KYNURENINE PATHWAY

Submitted by - student name



REGISTRATION NUMBER - xxxxxx

INTRODUCTION

The enzyme formamidase catalyzed the chemical reaction between formamide and water. This enzyme belongs to the hydrolase family. Despite the fact that the KYNFA enzymatic activity (also called as aryl formamidase) linked with the second stage of the kyn pathway was identified in early research. Also, the particular gene responsible for it was unknown for a very long time. Recently, studies have shown that this is an enzyme that could be used in different species. (Sorci et al., 2010) The main function of formamidase is to catalyze the hydrolysis of N-formyl-L-kynurenine to L-kynurenin, the second step is tryptophan breakdown. Kynurenine can be oxidized further to form nicotinic acid, NADH and NADPH. The main pathways involved are the shikimate pathway and the kynurenine pathway. The enzyme commission number is EC 3.5.1.49 – formamidase. (Wu et al., 2005) The disease that formamidase causes is tuberculosis and other neurodegenerative diseases but in the recent studies there has not been much research going on about what type of diseases formamidase causes. (Dykhno et al., 1964) The pathways are poorly recognized in fungi and have more evidence shown in bacteria and mammals.

Akanthomyces lecanii belongs to the *Cordycipitaceae*, *Hypocreales*, *Sordariomycetes*, and *Ascomycota* families of fungi. *Cordyceps confragosa*, *Torrubiella confragosa*, *Lecanicillium lecanii*, and other names have been used in the past. This species is interesting because it may infect a variety of insects (especially scale insects), has a wide geographic distribution. This fungus also has anti-parasitic nematode and anti-fungal disease properties (Zhang et al., 2020). This strain was first isolated in China.

Protein localization and protein-protein interaction in a cell are directly linked to the function and knowing the subcellular location of proteins is very essential for obtaining a complete picture of the cells (Shekari et al., 2014). So, understanding the protein subcellular localization is very important for both the function of the protein and the overall organization of the cell. The localization can easily be seen in large number of proteins (Scott et al., 2005). There are three main reasons why protein-protein interaction is important. a) The function and behavior of the protein can be understood, b) Prediction of the biological processes that are not known can be retrieved, c) The characteristics of protein complexes and the pathways can be retrieved or also can be used as a draft map. Understanding how gene functions and regulatory are integrated at the organismal level requires studying the interactome, which is the entire network of molecular physical interactions between biological entities in cells and organisms (Sevimoglu & Arga, 2014). Hence, protein-protein interaction and protein localization give more insight on the pathway that formamidase enzyme is involved in. Therefore, the research question that the report aims to solve: **Studying the protein-protein interaction of formamidase in *Akanthomyces lecanii* and understanding the reasoning behind them.**

METHODOLOGY

ASSEMBLY OF SEQUENCE - The two reads, forward and reverse transcript were downloaded from the directory and then uploaded in the Galaxy server and were assembled to contigs using genome assembler (Version 1.0) (Bankevich et al., 2012) with default parameters.

DNA ANNOTATION - The reference transcript was used to search against the protein database using BLASTX (Altschul et al., 1990) to give the protein of interest, and while doing the process in blastx, the default parameters were used. The protein was selected on the criteria of having the highest identity percentage and E value. BLOSUM62 matrix was used as a scoring matrix for building query from BLAST search.

PROTEIN DOMAINS – The protein sequence was uploaded to protein domain database search engines like PFAM (Version 34.0) (Mistry et al., 2021) and PROSITE (Release 2021_03 of 02-Jun-2021) (Sigrist et al., 2010) separately with default search parameters.

TOPOLOGICAL SIGNALS AND PROTEIN LOCALISATION - Signal IP (version 5.0) (Almagro Armenteros et al., 2019) was used to predict the presence of signal peptides and the location of their cleavage sites in proteins. Fungi group was chosen and other parameters were set to default. Protein localization was predicted using WoLF PSORT (Horton et al., 2007) and Deeploc (v1.0) (Almagro Armenteros et al., 2017). The organism for these tools was set to fungi and the rest were default parameters. TMHMM (version 2.0) was used for the prediction of transmembrane helices in protein. String-db (version 11.5) (Szklarczyk et al., 2021) was used to find the protein-protein interactions.

MULTIPLE SEQUENCE ALIGNMENT AND PHYLOGENETIC TREE - The multiple sequence alignment and Phylogenetic tree were assessed using ClustalX (2.1)(Larkin et al., 2007) and FigTree (version 1.4.4) (FigTree,07/10/2021). Two trees were built, one for closely related species and one for distantly related species. Firstly, a BLAST search was done to find the homologous sequences. For distantly related species Uniprot/Swissprot was used and for closely related species protein nr database was used. For distantly related sequences, hits > 50% similar was used and for closely related sequences hits > 80% similarity were chosen. For the phylogenetic analysis a bootstrap NJ tree was built with the output format of bootstrap labels that were changed to node. The tree was then visualized using FigTree with node labels and bootstrap values.

HOMOLOGY MODELLING AND QUALITY CHECK - Due to the low E value (0) and high GA341 score (>0.7), models based on templates 2wkn.1. A was chosen from the Swissmodel (Waterhouse et al., 2018) website. There was only one model based on the template. The models were then downloaded and their appropriate PDB templates files were used. Using Pymol (V2.5.2 super) function alignment query, the template and model were visually and structurally aligned. ProSA (Wiederstein & Sippl, 2007) web tools and PROCHECK (Laskowski et al., 1993) web tools were used to do quality checks, and Ramachandran plots were drawn using default settings using ProSA web tools and PROCHECK web tools.

RESULT AND DISCUSSION

DNA ASSEMBLY:

The forward and reverse reads were assembled using SPADES genome assembler. These reads were then assembled into a single contig of length 1234 nucleotides and the coverage of the assembly was 12.5454. Manually the calculations are shown in appendix (1)

DNA ANNOTATION USING BLASTX: The assembled sequence was then uploaded to BLASTX tool and the search was done with default parameters. The best hit matched the formamidase enzyme that belonged to the organism *Aknathomyces lecanii* which had a bit score value of 850 and an E value of 0 and has a similarity of 100%. The protein was 412 amino acids long.

DOMAIN IDENTIFICATION AND SIGNAL PEPTIDE:

DOMAIN IDENTIFICATION:

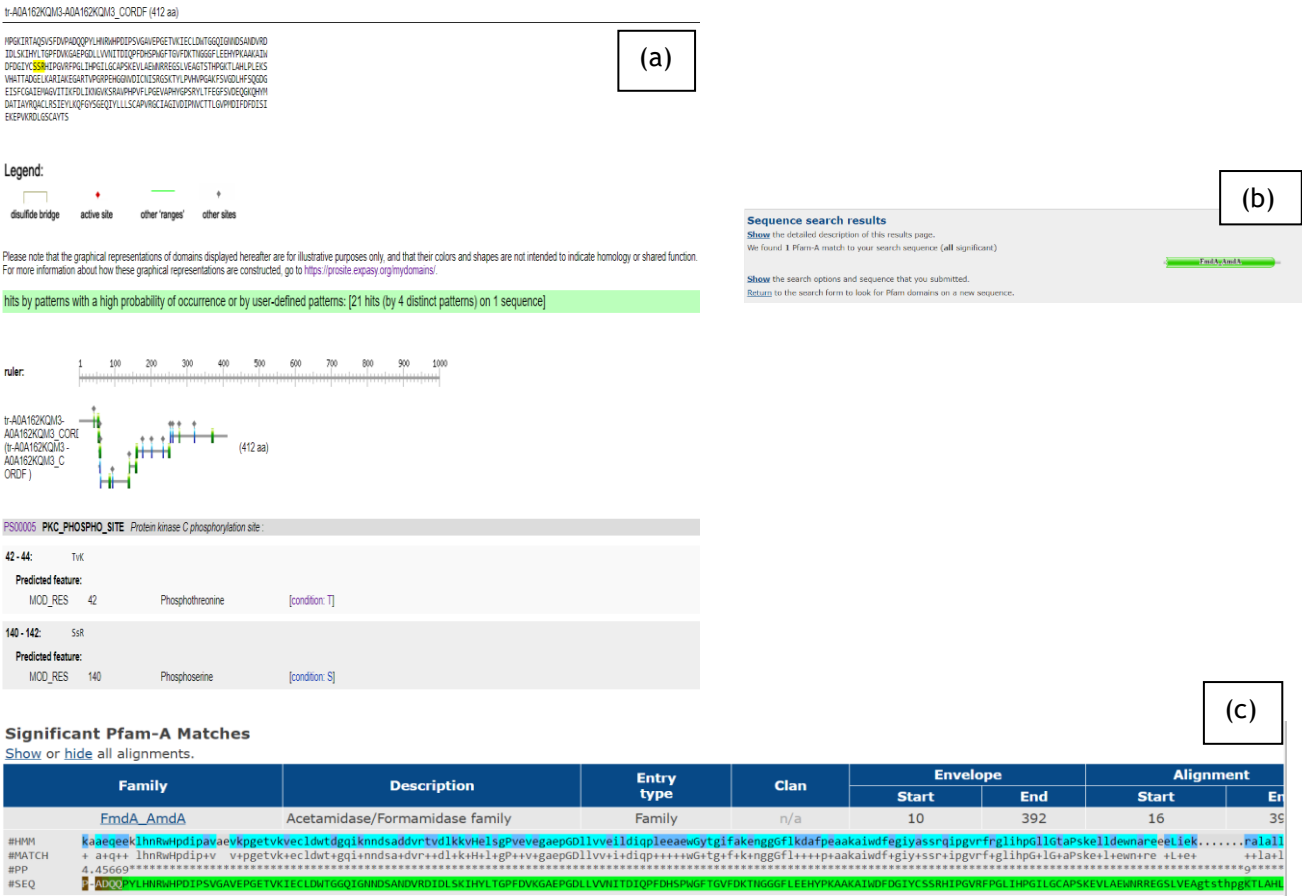


Fig1: a – Result page of prosite where 21 hits by 4 pattern hits were found. b & c – Results of significant domains obtained from Pfam.

There were no hits found on SMART. The PROSITE found 21 hits of the protein domain kinase C-phosphorylation site (location 42-44) and also many different domains like the N-myristylation site, N-glycosylation site were found. The Pfam predicted the Acetamidase/formamidase family which had a Bit score value of 584.0 and the alignment location is from 16-392.

PROTEIN LOCALISATION AND SIGNAL PEPTIDE

165073481328764 WoLFPSORT prediction cyto: 21.5, cyto_nucl: 12.333, cyto_pero: 12.166

27 Nearest Neighbors					
id	site	distance	identity	comments	
FKBP_YEAST	cyto	288.545	11.4078%	[Uniprot]	SWISS-PROT45 Cytoplasmic. GO:0005634; C:nucleus; Evidence:IDA
AMP1_YEAST	vacu	303.933	14.5914%	[Uniprot]	SWISS-PROT45: Vacuolar. GO:0003224; C:vacuole (sensu Fungi); Evidence:IDA
SODC_CANAL	cyto	307.042	10.9223%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
OAT_YEAST	cyto	323.024	13.8889%	[Uniprot]	SWISS-PROT45: Cytoplasmic. GO:0005634; C:nucleus; Evidence:IDA
PGM2_YEAST	cyto	336.195	15.993%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
IDHC_YEAST	cyto	337.184	11.6667%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
PGM1_YEAST	cyto	349.323	15.9649%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
SODC_NEUCR	cyto	354.201	12.376%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
IDH2_CANTR	pero	355.888	10.9005%	[Uniprot]	SWISS-PROT45: Peroxisomal.
PSB6_YEAST	cyto_nucl	360.254	14.0777%	[Uniprot]	SWISS-PROT45: Cytoplasmic and nuclear
EF1A_AJECA	cyto	365.234	14.8387%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
ADH2_KLUMA	cyto	366.192	15.0485%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
HOSM_YARLI	mito	368.236	11.6331%	[Uniprot]	SWISS-PROT45: Mitochondrial.
DYLL1_YEAST	cyto	368.301	6.79612%	[Uniprot]	SWISS-PROT45: Cytoplasmic. GO:0005623; C:cell; Evidence:NAS
GLNA_AGABI	cyto	369.271	14.3204%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
ADH1_KLUMA	cyto	378.697	16.9903%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
EF1A_ASPOB	cyto	382.74	13.6659%	[Uniprot]	SWISS-PROT45: Cytoplasmic.
PSB1_YEAST	cyto_nucl	385.794	15.0485%	[Uniprot]	SWISS-PROT45: Cytoplasmic and nuclear
VAOX_PENSI	cyto_pero	386.136	13.3929%	[Uniprot]	SWISS-PROT45: Peroxisomal and cytoplasmic.

Predicted proteins

tr_A0A162KQM3_A0A162KQM3_CORDF

Prediction: Cytoplasm, Soluble

Localization	Cytoplasm	Peroxisome	Extracellular	Plastid	Mitochondrion	Nucleus	Endoplasmic reticulum	Lysosome/Vacuole	Cell membrane
Likelihood	0.6992	0.2444	0.0186	0.01	0.0093	0.0093	0.0035	0.003	0.0025
Type	Soluble	Membrane							
Likelihood	0.9929	0.0071							

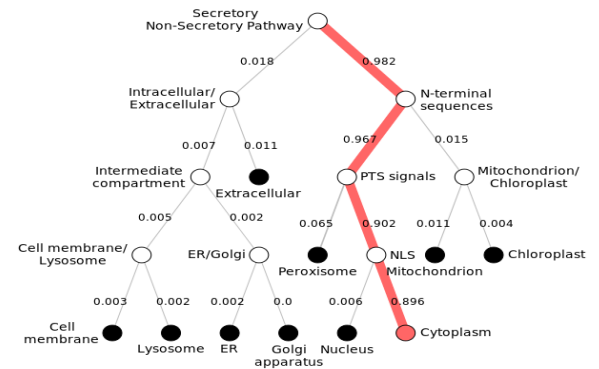


Fig2: The protein localization was determined by WoLF PSORT and DeepLoc tools. The fig. to the left shows that WoLF PSORT finds the nearest neighbors that are preset in the cytoplasm. The figure to the right presents the DeepLoc results which shows that the protein has a likelihood to localize in the cytoplasm.

Signal IP found no signal peptides and TMHMM also did not show presence of any transmembrane domains. By using the protein localization tools like WoLF PSORT and DeepLoc was found that the protein has the highest probability of localizing in the cytoplasm with a likelihood of 0.6992

To show protein localization of the enzymes and to show the protein-protein interaction between the tool String-db was used. The enzymes selected for this study were based on the location which was found to be in the cytoplasm. (table1)

Protein Name	Accession number/Identifier
1) Glutamine Synthetase	A0A168HKS7
2) Malic enzyme	A0A168DVR3
3) Fumarate hydratase	A0A168FIL1
4) Nitrite reductase	A0A162LR72
5) Phosphoenolpyruvate carboxykinase	A0A162KSX6

Table1- Enzymes from the String-db

(1) WoLF PSORT Prediction

k used for kNN is: 27
165072550628764 details mito: 17.5, cyto_mito: 11.5, pero: 5, cyto: 4.5

(2) WoLF PSORT Prediction

k used for kNN is: 27
165072566928763 details mito: 11, cyto: 7.5, cyto_nucl: 7, nucl: 5.5

(5) **WoLF PSORT Prediction**

(4)

k used for *kNN* is: 27

ito: 2

165072578628763 [details](#) cyto: 12, cyto_nucl: 8.833, cyto_pero: 8.833, mito: 6, nucl: 4.5, pero: 4.5

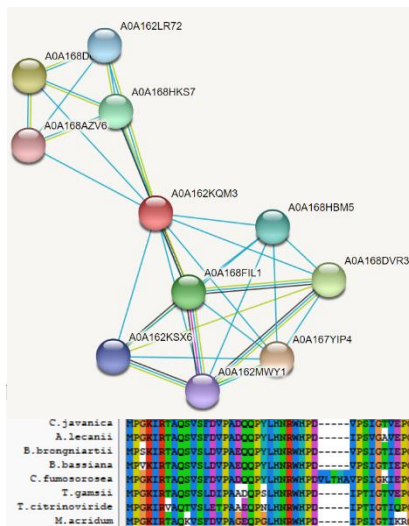


Fig3: 1,2,3,4 – They show the results of the location different enzymes mentioned in the table 1. (cytoplasm). Bottom – Shows the structure of the protein-protein interaction with the different enzymes that have interaction from the curated database.

MULTIPLE SEQUENCE ALIGNMENT AND PHYLOGENETIC TREE

Closely related species:

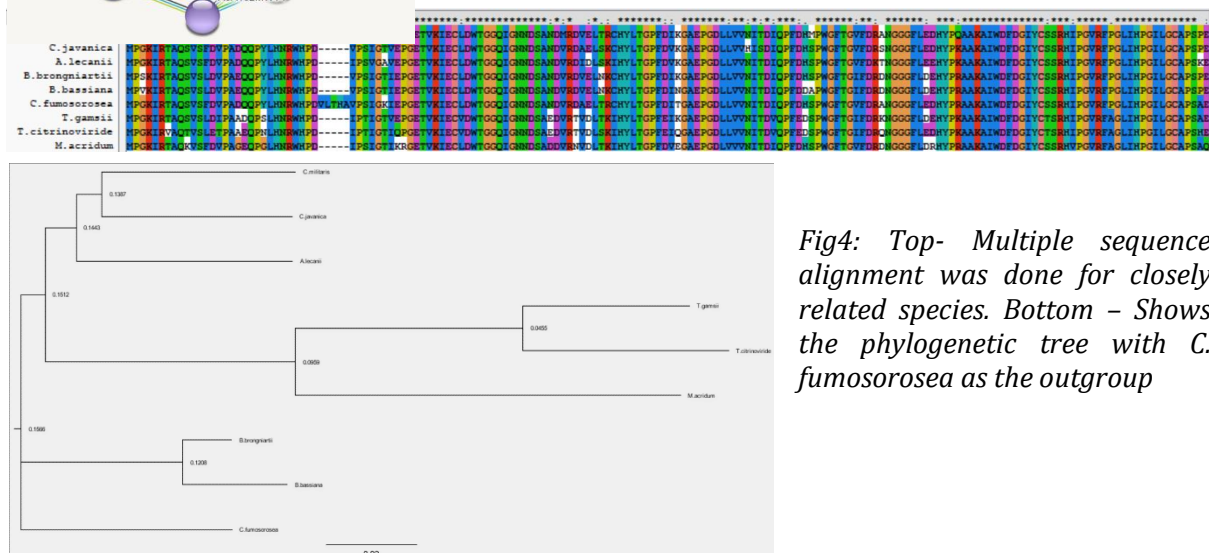
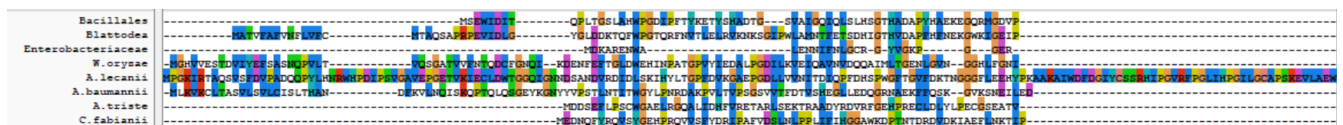


Fig4: Top- Multiple sequence alignment was done for closely related species. Bottom – Shows the phylogenetic tree with *C. fumosorosea* as the outgroup

Distantly related species:



DISCUSSION

DNA ASSEMBLY:

The estimated coverage for the gene length was found to be 43.176. This is very high when compared to the actual coverage of SPADES (12.5454). The estimated number of contigs for the coverage of 12.5454 was found to be $4.071775763 \times 10^{-27}$ contigs which is close to zero and not possible score. Hence, we get a single contig for this coverage. The calculation is shown in appendix (1)

DNA ANNOTATION:

BlastX was used to annotate and the best hit was matched with formamidase enzyme of the *Akanthomyces lecanii* with a 100% identity. The bit score and E value were 850 and 0 respectively. BlastX was the basis to annotate the respective raw reads that were given initially.

PROTEIN LOCALISATION AND SIGNAL PEPTIDE

The SMART tool for domain did not give any results maybe because it had only limited set of curated domains. PROSITE and Pfam found the presence of protein kinase phosphorylation site with 20 other hits against 4 patterns with a region between 42-44 which means that they have smaller database of patterns and profiles. PROSITE also found that protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues that are found close to C terminal. The results from Pfam had a bit score value of 584 with a region between 16-392. These results showed that they have a larger database patterns and profiles. Also, this particular domain predicted that it hydrolyzes formamide with the production of ammonia which can be used as a nitrogen source.

The protein localization results of WoLF PSORT showed the most of the sequences was soluble and found to localize mostly in the cytoplasm. The results of DeepLoc also gave similar results as WoLF PSORT.

This related back to my research question where it has shown that formamidase has protein interactions with the cell wall membrane proteins and cytosolic proteins because it is present in the fungus cytoplasm and cell wall and these interactions might be due to potential roles in the nitrogen pathway which was confirmed by the tool String.

MULTIPLE SEQUENCE ALIGNMENT AND PHYLOGENY

The *Akanthomyces lecanii* was aligned with other organisms of the same family. Hence, the results showed a close relation with species of the fungus family but showed distant relation with other organisms that were taken from Uniprot. These organisms were from the family of bacteria.

The node length of closely related species showed better results than distantly related species. The outlier that was obtained in closely related species was *C. fumosorosea* and there were two outliers in the distantly related species that were *A. triste* and *C. fabianii*. The tree of both closely related species and distantly related species showed distinct orthologs and paralogs with the other organisms. The table of organisms is shown in appendix (2)

3D MODELLING AND QUALITY CHECK

Through the quality check the model was found to be the optimum with a GMQE value of 0.85 which is greater than 0.7. The template and model were aligned with RMSD value of 0.174 >0.7 which means that the distance between the template and model was 0.174 Units away from the alignment. The Ramachandran plot indicated that a greater number of residues were present in the allowed regions. The PROSA showed a Z-score which was within the optimum region of z-scores. The local model quality of both the model and template was almost similar but the model graph showed a few peaks in the negative region.

RESEARCH QUESTION

The research question revolves around “Studying the protein-protein interaction of formamidase in *Akanthomyces lecanii* and understanding the reason behind it.”

To tackle the question, the tool string results showed that formamidase interacts with Malic enzyme, Glutamate synthetase, Phosphoenolpyruvate carboxykinase (ATP), Nitrite reductase and fumarate hydratase all of which had a homology score of >0.90 which is optimal. Most of these proteins had interactions from the curated databases (shown in blue). This network has significantly more interactions than expected which means that they are biologically connected. Therefore, the protein has mainly interactions with cell wall membrane proteins and cytosolic proteins as it is present in the fungus cytoplasm and cell wall and these interactions might be due to potential roles in the nitrogen metabolism pathway. In future, there could be research done on other interactions related to formamidase. It was experimentally proven that by isolating a cell wall protein interacted with formamidase at the fungal surface.

ABSTRACT

Formamidase is an enzyme of the hydrolase family which is associated with the Kynurenine pathway. Members of the fungi family such as the *Akanthomyces lecanii* are involved in this pathway as either an antifungal or as a pest for insects. As the location of the species is very important to find out the function and the pathway involved. Experiments have shown that this fungus is located mostly in the cytoplasm of the cell or in the mitochondria. A protein-protein interaction studies/experiment revealed that *Akanthomyces lecanii* has interaction with a few species which are also located in the cytoplasm such as the Glutamine synthetase, Malic enzyme, Fumarate hydratase, Nitrite reductase, Phosphoenolpyruvate carboxykinase. The interaction between these organisms had been derived from curated databases. The likelihood of the location of the protein was also found to be within the range. Therefore, in this research it was found that it mainly has interactions with cell wall membrane proteins and cytosolic proteins as its present in the fungus cytoplasm and cell wall and these interactions might be due to potential roles in the nitrogen metabolism pathway.

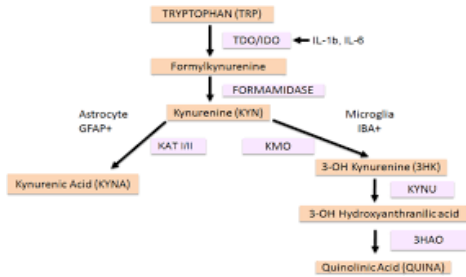
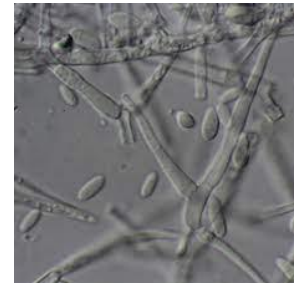


Fig7: Left – Kynurenine pathway.
Right – Microscopic view of *Aknathomyces lecanii*



APPENDIX

1) COVERAGE CALCULATION

Gene Length – 1234 ; No. of reads – 444 ; Length of each read – 60

Statistical coverage = $(444 \times 2 \times 60) / 1234 = 43.176$

2) SPECIES Table

Distantly related species	Closely related species
1) <i>Aknathomyces lecanii</i>	1) <i>Akanthomyces lecanii</i>
2) <i>Acinetobacter baumannii</i>	2) <i>Cordyceps militaris</i>
3) <i>Amblyomma triste</i>	3) <i>Beauveria brongniartii</i>
4) <i>Cyberlindnera fabianii</i>	4) <i>Cordyceps javanica</i>
5) <i>Bacillales</i>	5) <i>Cordyceps fumosorose</i>
6) <i>Blattodea</i>	6) <i>Beauveria bassiana</i>
7) <i>Enterobacteriaceae</i>	7) <i>Trichoderma gamsii</i>
8) <i>Weissella Oryzae</i>	8) <i>Metarhizium acridum</i>
	9) <i>Trichoderma citrinoviride</i>

3) GALAXY RESULT

Analyze Data

Tools

spades

×

1

2

3

#name

length

coverage

NODE_1

1234

12.5454

4) SMART RESULT

SMART

SETUP FAQ ABOUT GLOSSARY WHAT'S NEW FEEDBACK

Domains within *Cordyceps confragosa* RCEF 1005 protein A0A162KQM3_CORDF (A0A162KQM3)

Formamidase

SAVE

Information

Length 412 aa

Source database UniProt

Identifiers A0A162KQM3_CORDF, A0A162KQM3

The SMART diagram above represents a summary of the results shown below. Domains with scores less significant than established cutoffs are not shown in the diagram. Features are also not shown when two or more occupy the same piece of sequence; the priority for display is given by SMART > PFAM > PROSPERO repeats > Signal peptide > Transmembrane > Coiled coil > Unstructured regions > Low complexity. In either case, features not shown in the above diagram are marked as 'overlap' in the right side table below.

Confidently predicted domains, repeats, motifs and features:

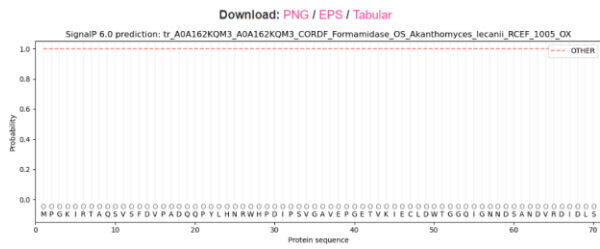
No domains, repeats, motifs or features could be predicted with confidence.

Features NOT shown in the diagram:

There are no hidden domains or features present.

5) SIGNAL IP RESULT

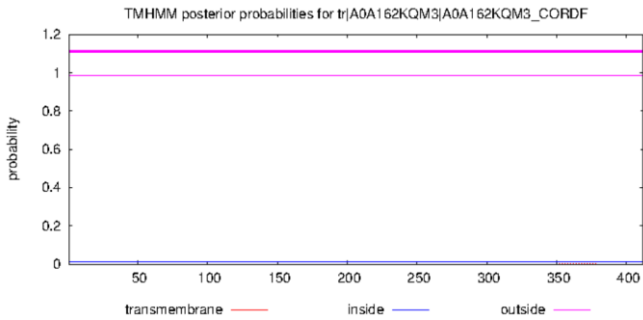
Protein type	Other	Signal Peptide (Sec/SPI)
Likelihood	1	0.0001



6) TMHMM RESULT

TMHMM result

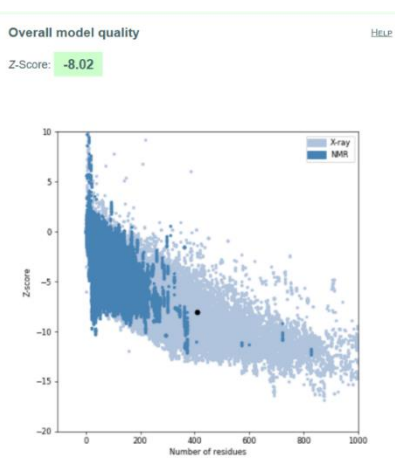
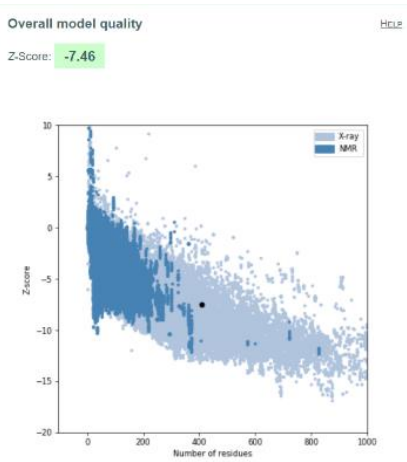
```
# tr|A0A162KQM3|A0A162KQM3_CORDF Length: 412
# tr|A0A162KQM3|A0A162KQM3_CORDF Number of predicted TMHs: 0
# tr|A0A162KQM3|A0A162KQM3_CORDF Exp number of AAs in TMHs: 0.03539
# tr|A0A162KQM3|A0A162KQM3_CORDF Exp number, first 60 AAs: 0
# tr|A0A162KQM3|A0A162KQM3_CORDF Total prob of N-in: 0.01387
tr|A0A162KQM3|A0A162KQM3_CORDF TMHMM2.0 outside 1 412
```



7) Overall Model Quality

Model

Template



8) Blast Search sequences

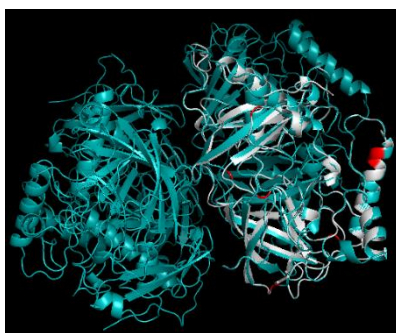
Sequences producing significant alignments			Download ▾	Select columns ▾	Show 100 ▾	?			
<input checked="" type="checkbox"/> select all	100 sequences selected	GenPept	Graphics	Distance tree	Multiple alignment	MSA Viewer			
<input checked="" type="checkbox"/>	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Id	Per Len	Accession
<input checked="" type="checkbox"/>	formosensis [Acanthomyces islandi RCEP 3165]	Acanthomyces islandi RCEP 3165	805	856	100%	0	100%	100%	QAA2448
<input checked="" type="checkbox"/>	formosensis [Corynebacterium milliae]	Corynebacterium milliae	793	793	100%	0	100.00%	100%	ZV163424.1
<input checked="" type="checkbox"/>	formosensis [Corynebacterium millae] CM011	Corynebacterium millae CM011	792	792	100%	0	100.00%	100%	XP_006669077.1
<input checked="" type="checkbox"/>	formosensis [Corynebacterium islandicum]	Corynebacterium islandicum	791	791	100%	0	100.00%	100%	ZJ295148.1
<input checked="" type="checkbox"/>	formosensis [Beauveria longirostris RCEP 3172]	Beauveria longirostris RCEP 3172	778	778	100%	0	89.32%	100%	QAA38182.1
<input checked="" type="checkbox"/>	formosensis [Corynebacterium formosense RCEP 2673]	Corynebacterium formosense RCEP 2673	771	771	100%	0	88.81%	100%	XP_018706293.1
<input checked="" type="checkbox"/>	notulae formosensis [Beauveria basinalis]	Beauveria basinalis	769	769	100%	0	88.11%	100%	D882115.1
<input checked="" type="checkbox"/>	biochemical screen: [H0105_000 (H1230 Beauveria basinalis)]	Beauveria basinalis	769	769	100%	0	88.11%	100%	D264139.1
<input checked="" type="checkbox"/>	formosensis [Beauveria basinalis RCEP 2466]	Beauveria basinalis RCEP 2466	764	764	100%	0	87.62%	100%	XP_005598323.1
<input checked="" type="checkbox"/>	formosensis [Corynebacterium millae] CM001	Corynebacterium millae CM001	738	738	100%	0	66.17%	100%	XP_006647612.1
<input checked="" type="checkbox"/>	formosensis [Corynebacterium millae]	Corynebacterium millae	736	736	100%	0	65.92%	100%	AF161182.1
<input checked="" type="checkbox"/>	biochemical screen: [G140406_01 (H1231 Trichoderma gamsii)]	Trichoderma gamsii	716	716	100%	0	81.31%	100%	D294168.1
<input checked="" type="checkbox"/>	formosensis [Trichoderma gamsii]	Trichoderma gamsii	714	714	100%	0	81.67%	100%	XP_016666027.1
<input checked="" type="checkbox"/>	biochemical protein: [Z459_022233 Trichoderma arundinis]	Trichoderma arundinis	714	714	100%	0	81.37%	100%	K05845598.1
<input checked="" type="checkbox"/>	biochemical screen: [H1_0159_004848 Trichoderma reesei]	Trichoderma reesei	713	713	100%	0	89.10%	100%	BA055222.1
<input checked="" type="checkbox"/>	biochemical screen: [formosensis [Trichoderma discoloris]]	Trichoderma discoloris	713	713	100%	0	89.34%	100%	XP_00592591.1
<input checked="" type="checkbox"/>	biochemical screen: [H10176_01_20752 Trichoderma harzianum]	Trichoderma harzianum	713	713	100%	0	88.9%	100%	XP055659.1
<input checked="" type="checkbox"/>	biochemical screen: [H10176_01_20752 Trichoderma harzianum] CS 226 95	Trichoderma harzianum CS 226 95	713	713	100%	0	88.9%	100%	XP_007377493.1
<input checked="" type="checkbox"/>	biochemical screen: [H10176_01_20752 Trichoderma harzianum] [H10176_01_20752]	Trichoderma harzianum [H10176_01_20752]	713	713	100%	0	77.96%	100%	Z64656264.1
<input checked="" type="checkbox"/>	biochemical screen: [Z228_095622 Trichoderma reesei]	Trichoderma reesei	712	712	100%	0	81.62%	100%	Z1832228.1
<input checked="" type="checkbox"/>	formosensis [Beauveria longirostris RCEP 3173]	Beauveria longirostris RCEP 3173	713	713	100%	0	80.60%	100%	XP_010728119.1
<input checked="" type="checkbox"/>	formosensis [Beauveria longirostris RCEP 3172]	Beauveria longirostris	711	711	100%	0	80.10%	100%	XP_00737921.1
<input checked="" type="checkbox"/>	biochemical screen: [H2123_000203 Trichoderma reesei]	Trichoderma reesei	711	711	100%	0	79.85%	100%	Z1051849.1
<input checked="" type="checkbox"/>	formosensis [Beauveria longirostris RCEP 3172]	Trichoderma discoloris	711	711	100%	0	89.34%	100%	XP_01294361.1

✓	formosensis strain D2003_2393 (Coffea arabica, BAQ-2017)	Coffea arabica, BAQ-2017	705	705	99%	0	80.88%	43	PH930202.1
✓	formosensis (Mucorales, Rhiz. RCEP-2458)	Mucorales, Rhiz. RCEP-2458	704	705	100%	0	80.15%	46	KZ290911.4
✓	Antennariae/formosensis (Fusarium solani)	Fusarium solani	704	704	99%	0	80.15%	42	XP_048611173.1
✓	putative formosensis (Fusarium korributhi)	Fusarium korributhi	704	704	99%	0	80.15%	42	BM627566.1
✓	formosensis (Metarhizium antioxiense)	Metarhizium antioxiense	703	703	99%	0	80.15%	43	XP_G84373.1
✓	formosensis formosensis (Fusarium sp., AF_6)	Fusarium sp., AF_6	702	702	99%	0	79.90%	42	ISLH08001
✓	broedheadii strain TC31_02126 (Tichodroma cinnam. domes)	Tichodroma cinnam. domes	702	702	100%	0	77.25%	43	KAH95094.1
✓	Putative Formosensis (Tornaliella hennelae)	Tornaliella hennelae	702	702	99%	0	80.68%	44	CEJ03055.1
✓	formosensis (Fusarium sporisoridis)	Fusarium sporisoridis	701	701	99%	0	77.78%	42	RGF5094.1
✓	uncharacterized strain TORICRAFT_01511 (Tichodroma ressei CM6)	Tichodroma ressei CM6	701	701	100%	0	76.64%	43	XP_006969739.1
✓	broedheadii strain CP32_1273 (Ophiocordyceps cameroni leonardi (non. inal.))	Ophiocordyceps cameroni leonardi (non. inal.)	701	701	99%	0	79.08%	42	RD40665.1
✓	formosensis (Tichodroma aeneae)	Tichodroma aeneae	701	701	100%	0	78.40%	43	TO450535.1
✓	uncharacterized strain FV9085_1391 (Fusarium venenatum)	Fusarium venenatum	700	700	100%	0	79.68%	42	XP_025436068
✓	putative formosensis CM69.54 (Fusarium asenellum)	Fusarium asenellum	699	699	100%	0	77.28%	42	GFPS596.1
✓	uncharacterized strain FJESCA_06729 (Fusarium coffeae)	Fusarium coffeae	698	698	100%	0	78.01%	42	XP_031016231
✓	broedheadii strain FSC_3632 (Fusarium decemcoloratum)	Fusarium decemcoloratum	698	698	99%	0	79.17%	42	KAF336921.1
✓	formosensis strain F5465_3467 (Fusarium saccharorum)	Fusarium saccharorum	698	698	99%	0	77.96%	42	KGF099212.1
✓	formosensis (Metarhizium asenellum GMA 152)	Metarhizium asenellum GMA 152	698	698	99%	0	78.76%	42	XP_003137178
✓	Antennariae/formosensis (M. MPS-SQER-AT-0672)	Fusarium sp. MPS-SQER-AT-0672	697	697	99%	0	76.60%	43	KAH95094.1
✓	Antennariae/formosensis (M. MPS-SQER-0278)	Fusarium sp. MPS-SQER-0278	697	697	99%	0	76.60%	43	KAF653996.1
✓	broedheadii strain L39_0172 (Ophiocordyceps schizophyae farsae BCC-5412)	Ophiocordyceps schizophyae farsae BCC-5412	697	697	99%	0	76.97%	42	BC121116.1
✓	formosensis strain TORICRAFT_014245 (Tichodroma virens Gu-28)	Tichodroma virens Gu-28	697	697	100%	0	76.61%	43	XP_013961196
✓	formosensis (Fusarium collinum)	Fusarium collinum	696	696	99%	0	79.66%	40	ASD4293163.1
✓	Antennariae/formosensis (Fusarium bicoloratum)	Fusarium bicoloratum	696	696	98%	0	79.75%	41	KAH7262765.1
✓	erobolae formosensis (Fusarium collinum ET1)	Fusarium collinum ET1	695	695	99%	0	79.66%	40	XP_013101127
✓	formosensis (Fusarium verticillioides 7550)	Fusarium verticillioides 7550	695	695	99%	0	79.66%	40	XP_018752122
✓	putative formosensis (Fusarium mangroveae)	Fusarium mangroveae	695	695	99%	0	79.41%	40	XP_041656033
✓	formosensis (Fusarium pseudosporisorium)	Fusarium pseudosporisorium	695	695	99%	0	79.66%	40	KAF57521212.1
✓	formosensis (Fusarium arbusculum)	Fusarium arbusculum	695	695	99%	0	79.66%	40	KAF548014.1

✓	hypothetical protein TA0571_041 (Fusarium oxysporum)	<i>Fusarium oxysporum</i>	711	710	99%	0.03876	412	K05225066.1
✓	hypothetical protein F087_04402 (Fusarium sp. FJSC-9/R)	<i>Fusarium sp. FJSC-9/R</i>	710	710	99%	0.03876	413	K01055500.1
✓	formamide (Metachlorella bionum ARS27_3207)	<i>Metachlorella bionum ARS27_3207</i>	710	710	99%	0.03906	413	XP_014558693.1
✓	hypothetical protein HY566_02209 (Fusarium oxysporum)	<i>Fusarium oxysporum</i>	710	710	99%	0.03972	412	QC7274.1
✓	Defective formamide (Metachlorella bionum ARS27_3209)	<i>Metachlorella bionum ARS27_3209</i>	710	710	99%	0.03949	413	K080351.1
✓	acetic acid/Formamide (Trichoderma aspergilloides)	<i>Trichoderma aspergilloides</i>	710	710	100%	0.06165	413	K048727022.1
✓	putative formamide (Fusarium solani)	<i>Fusarium solani</i>	709	709	99%	0.03976	412	P103202.1
✓	formamide (Fusarium oxysporum F041)	<i>Fusarium oxysporum F041</i>	709	709	99%	0.03876	412	XP_011323254.1
✓	acetic acid/Formamide (Fusarium fupiformis)	<i>Fusarium fupiformis</i>	708	708	99%	0.04078	412	XP_048767890.1
✓	hypothetical protein F196_07275 (Fusarium oxysporum/graminis CS3296)	<i>Fusarium oxysporum/graminis CS3296</i>	709	709	99%	0.03876	412	XP_00528718.1
✓	hypothetical protein MH0001_01873 (Metachlorella bionum)	<i>Metachlorella bionum</i>	709	709	99%	0.06449	413	K04900877.1
✓	hypothetical protein J3458_00440 (Metachlorella arundinis)	<i>Metachlorella arundinis</i>	708	708	99%	0.06084	413	K048841568.1
✓	formamide (Metachlorella wuhoensis ARS27_327)	<i>Metachlorella wuhoensis ARS27_327</i>	709	709	99%	0.06449	413	K050684.1
✓	putative formamide (Metachlorella arundinis)	<i>Metachlorella arundinis</i>	708	708	99%	0.03906	413	K07513400.1
✓	formamide (Trichoderma harzianum)	<i>Trichoderma harzianum</i>	709	709	100%	0.08610	413	K0927463.1
✓	unannoted protein product (Fusarium graminearum)	<i>Fusarium graminearum</i>	709	709	99%	0.06126	412	CAG261246.1
✓	unannoted protein product (Fusarium graminearum)	<i>Fusarium graminearum</i>	708	708	99%	0.06136	412	CAC364700.1
✓	valine/Formamide (Fusarium sp. JF-3)	<i>Fusarium sp. JF-3</i>	708	708	99%	0.06136	412	BL344596.1
✓	hypothetical protein MAT0709_00024 (Trichoderma aspergilloides CBS 433.97)	<i>Trichoderma aspergilloides CBS 433.97</i>	708	708	100%	0.06193	413	XP_034794303.1
✓	hypothetical protein J0881_000268 (Trichoderma vanderii)	<i>Trichoderma vanderii</i>	707	707	99%	0.07806	413	XP_004594303.1
✓	hypothetical protein J0881_000269 (Trichoderma vanderii)	<i>Trichoderma vanderii</i>	708	708	99%	0.06193	412	XP_004594303.1
✓	hypothetical protein J0881_011021 (Trichoderma vanderii)	<i>Trichoderma vanderii</i>	708	708	99%	0.06193	412	XP_004594303.1
✓	hypothetical protein J0881_011022 (Trichoderma vanderii)	<i>Trichoderma vanderii</i>	708	708	99%	0.06193	412	XP_004594303.1
✓	hypothetical protein V5502_101566 (Pachystima chrysodendroides 170)	<i>Pachystima chrysodendroides 170</i>	707	707	99%	0.07617	413	XP_011874737.1
✓	hypothetical protein V5502_101566 (Pachystima chrysodendroides 170)	<i>Pachystima chrysodendroides 170</i>	707	707	99%	0.08148	401	K023845.1
✓	unannoted protein product (Fusarium sp.)	<i>Fusarium sp.</i>	707	707	99%	0.03906	412	K04751054.1
✓	hypothetical protein CP033_26146 (Hyphocorynebacterium campodori-sandneri (nom. nov.))	<i>Hyphocorynebacterium campodori-sandneri (nom. nov.)</i>	706	706	99%	0.03996	412	RE456055.1
✓	putative formamide (Fusarium avenaceum)	<i>Fusarium avenaceum</i>	706	706	99%	0.06449	412	RE112946.1
✓	formamide (Metachlorella arundinis ARS27_3541)	<i>Metachlorella arundinis ARS27_3541</i>	705	705	99%	0.07175	413	XP_048767633.1

<input checked="" type="checkbox"/>	<i>Acetaminide</i> [Acetaminide] <i>CGMA.1021</i>	<i>Melanin</i> <i>acromin CGMA.102</i>	090	090	99%	0	73.76%	41	<i>XP_007813733.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [formamide] <i>Fusarium</i> <i>sp.</i> <i>MPH.SOFAT.AT.6072</i>	<i>Fusarium</i> <i>sp.</i> <i>MPH.SOFAT.AT.6072</i>	097	097	99%	0	70.90%	40	<i>XP_04744665.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>sp.</i> <i>NHRL.5270</i>]	<i>Fusarium</i> <i>sp.</i> <i>NHRL.5270</i>	097	097	99%	0	70.66%	40	<i>KAF5636906.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>L249_3712</i> [<i>Orthococcus polydactylus</i> <i>faculta</i> <i>BC.533.142</i>]	<i>Orthococcus polydactylus</i> <i>faculta</i> <i>BC...</i>	097	097	99%	0	71.87%	42	<i>RG121118.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>TRBQVAFAT_141248</i> [<i>Trichoderma</i> <i>sibiricum</i> <i>CG-9.8</i>]	<i>Trichoderma sibiricum</i> <i>CG-9.8</i>	097	097	100%	0	71.61%	43	<i>XP_013061195.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>affinis</i>]	<i>Fusarium affinis</i>	096	096	99%	0	70.66%	40	<i>KAG4529162.1</i>
<input checked="" type="checkbox"/>	<i>Acetaminide</i> [Fusarium <i>trichotum</i>]	<i>Fusarium trichotum</i>	096	096	98%	0	70.75%	41	<i>KAH7828795.1</i>
<input checked="" type="checkbox"/>	<i>asorbile</i> <i>formamide</i> [Fusarium <i>affinis</i> <i>formae</i> <i>ET.1</i>]	<i>Fusarium affinis</i> <i>formae</i> <i>ET.1</i>	095	095	99%	0	70.66%	40	<i>XP_010301275.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>verticilloides</i> <i>7601</i>]	<i>Fusarium verticilloides</i> <i>7601</i>	095	095	99%	0	70.66%	40	<i>XP_018782322.1</i>
<input checked="" type="checkbox"/>	<i>autotile</i> <i>formamide</i> [Fusarium <i>maioformis</i>]	<i>Fusarium maioformis</i>	095	095	99%	0	71.41%	40	<i>XP_010898357.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>pseudosanthobium</i>]	<i>Fusarium pseudosanthobium</i>	095	095	99%	0	70.66%	40	<i>KAF5627162.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>abhyobolus</i>]	<i>Fusarium abhyobolus</i>	095	095	99%	0	70.66%	40	<i>KAF5645354.1</i>
<input checked="" type="checkbox"/>	<i>Acetaminide</i> [Fusarium <i>redolens</i>]	<i>Fusarium redolens</i>	094	094	99%	0	71.41%	40	<i>XP_004016426.1</i>
<input checked="" type="checkbox"/>	<i>asorbile</i> <i>formamide</i> [Fusarium <i>fulvum</i> <i>IMI.5829</i>]	<i>Fusarium fulvum</i> <i>IMI.5829</i>	094	094	99%	0	71.41%	40	<i>XP_023429602.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>clavatum</i>]	<i>Fusarium clavatum</i>	094	094	99%	0	71.41%	40	<i>KAF5659935.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>FANTH_12935</i> [Fusarium <i>anthobolus</i>]	<i>Fusarium anthobolus</i>	094	094	99%	0	71.41%	40	<i>KAF5623118.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>dentificatum</i>]	<i>Fusarium dentificatum</i>	094	094	99%	0	71.41%	40	<i>KAF5680271.1</i>
<input checked="" type="checkbox"/>	<i>autotile</i> <i>formamide</i> [Fusarium <i>autroanthracium</i>]	<i>Fusarium autroanthracium</i>	094	094	99%	0	70.90%	40	<i>KAF5466127.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>naefome</i>]	<i>Fusarium naefome</i>	094	094	99%	0	71.41%	40	<i>KAF5630264.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>AU129_110116</i> [Fusarium <i>ovosporium</i> <i>sp.</i> <i>radici</i> <i>cosumetum</i>]	<i>Fusarium ovosporium</i> <i>sp.</i> <i>radici</i> <i>cosumetum</i>	094	094	99%	0	71.17%	40	<i>PCD03443.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>subolydus</i>]	<i>Fusarium subolydus</i>	094	094	99%	0	71.17%	40	<i>XP_036534265.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>PVER14953_36567</i> [Fusarium <i>verticilloides</i>]	<i>Fusarium verticilloides</i>	094	094	99%	0	71.41%	40	<i>BBQ77726.1</i>
<input checked="" type="checkbox"/>	<i>asorbile</i> <i>formamide</i> [Fusarium <i>fulvum</i>]	<i>Fusarium fulvum</i>	094	094	99%	0	71.17%	40	<i>SCV41414.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>meicium</i>]	<i>Fusarium meicium</i>	093	093	99%	0	78.92%	40	<i>KAF5645669.1</i>
<input checked="" type="checkbox"/>	<i>hydrothermal</i> <i>ecoli</i> <i>H9272_012023</i> [Fusarium <i>xyloroides</i>]	<i>Fusarium xyloroides</i>	093	093	99%	0	71.41%	40	<i>KAG579257.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>tiastiba</i>]	<i>Fusarium tiastiba</i>	093	093	99%	0	71.17%	40	<i>XP_037267664.1</i>
<input checked="" type="checkbox"/>	<i>formamide</i> [Fusarium <i>apagathi</i>]	<i>Fusarium apagathi</i>	093	093	99%	0	71.17%	40	<i>KAF468738.1</i>

9) 3D HOMOLLOGY MODELLING – Template (grey), Model (teal), active site residues (red)



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/2wkntemplate//A/2      6      11      16      21      26      31      36      41      46      51      56      61      66      71
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/model_01//A/5          11      16      21      26      31      36      41      46      51      56      61      66      71      76
TRTAGSVSDVDPADQDPVYLNHRWHPDTPSGGAVFPGETVKTICLDTWTGGGTGNGNDSDVDRDITLSTKTHYTGPF

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