Journal of Recent Advances in Applied Sciences

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Research Article

# *In-Silico* CHARACTERIZATION OF 14-ALPHA STEROL DEMETHYLASE OF *Aspergillus fumigatus*

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Received November 04, 2015; Accepted December 02, 2015; Published December 05, 2015;



**Cite This Article:** Verma, P., Yadav, N., Sarvendra, K., Srivastava, A.(2015). *In-Silico* Characterization of 14-alpha Sterol Demethylase of *Aspergillus fumigatus*. Journal of Recent Advances in Applied Sciences, 29(1).1-8

**ABSTRACT**

The eukaryotes sterol pathways are extremely conserved and these biosynthetic pathway are very long which includes the synthesis of dolichols, coenzyme Q, heme A, and isoprenylated proteins.14-Demethylase is an essential enzyme of the cytochrome P450 superfamily, which is potential the target of azole antifungals. Predicted results shows that 14-alpha sterol demethylase have molecular weight of 58930.8 Daltons and the theoretical isoelectric point (pI) of 7.64. The negative Grand average of hydropathicity (GRAVY) index of -0.125. The Aliphatic index of *Aspergillus fumigates* 14-alpha sterol demethylase is 89.48. Alpha helix (Hh) accounts 210 amino acids of about 40.08%. The extended strand (Ee) had 91 amino acids accounting 17.37, Beta turn (Tt) made up of 51 amino acids making up 9.73% and random coil (Cc) made up of 172 amino acids accounting 32.82%. The subcellular localization of 14-alpha sterol demethylase Cyp51B was predicted to be a Plasma membrane protein.

**KEYWORDS:** *Aspergillus fumigates,* ergosterol, azole, cytochrome P450

## INTRODUCTION

Fungi are responsible for common life warning diseases (Lass-Flцrl, Cornelia., 2009). Aspergillus infections are very serious cause of mortality and *Aspergillus fumigatus* is one of the most frequent airborne fungal agents causing infection worldwide (Kelly, Steven L., et al., 2003). Recently, it is reported that the existence of two genes which codes for two different 14α-demethylases in *A. fumigatus*, cyp51A and cyp51B. The mechanisms of resistance against azoles have been reported (Diaz-Guerra et al., 2003, Latgeґ, J.-P. et al., 1999). In the Netherlands, the rapid emergence of multi-azole resistance (MAR) was observed in *A. fumigates* since 1998 (Howard et al., 2009).The most effective, crucial, and widely used antifungal compounds is the azoles which target sterol 14 demethylase and hinder with ergosterol synthesis in the fungus (Kelly, Steven L., et al., 2003).Modifications in azole target enzyme 14-sterol demethylase (Cyp51A) or overexpression of this enzyme results in resistance which is the prime mechanisms found in *A. fumigates* azole resistance (Mellado, Emilia, et al., 2011). Antifungal compounds shows their activity via a variety of mechanisms and the case of itraconazole and azole derivatives which is involved by blocking the ergosterol biosynthesis pathway by inhibiting the fungal cytochrome P450 enzyme lanosterol demethylase, which results arrest of fungal growth (Lupetti et al, 2002).This is reported that 50 to 80 percent of azole resistance in human pathogens are due to mutations in the sterol demethylase gene (CYP51A) (Bueid, Ahmed, et al ., 2010, Cools, H. J., et al. , 2006, Cools et al ., 2008).

Protein structure and computational studies are important tools in the study of drug action and resistance. It is reported that CYP51A may encode the major sterol 14-demethylase enzyme which is required for growth (Warrilow, Andrew GS, et al., 2010). CYP51B is another isoform of Sterol 14-Demethylase (CYP51) but not well studied and characterized, so here we have characterized it *in silico* for further study.

## METHODS

### Sequence retrieval

The amino acid sequence of the 14-alpha sterol demethylase of *Aspergillus fumigates* was obtained from the sequence database of NCBI (http://www.ncbi.nlm.nih.gov/protein/XP_749134.1). In the present work we have focused on Physico-chemical characterization, Secondary structure prediction, Subcellular localization prediction (Hossain, Md Musharaf.,2012).

### Physico-chemical characterization

The various parameters like viz; values of theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (Gill SC et al, 1989) instability index (Guruprasad et al, 1990), aliphatic index (kai AJ 1980) and grand average hydropathy (GRAVY) (Kyte J, 1982, Gupta, Sachin, et al. 2013) were computed.

### Secondary structure prediction

The secondary structure predictions have been carried out by the self optimized prediction method (SOPM) server with output width 70, the sequence of 14-alpha sterol demethylase of *Aspergillus fumigates* was submitted in the fasta formate and the results were analysed.

### Signal peptide prediction

SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) were used to predicts the presence and location of signal peptide cleavage sites in amino acid sequences 14-alpha sterol demethylase (Petersen et al, 2012).

### Subcellular localization prediction

Subcellular localization of 14-alpha sterol demethylase was predicted by CELLO v.2.5 (Lubec G., L. et al, 2005). Results were also cross-checked with another subcellular localization PredictProtein servers (Yu N. Y., et al, 2010, Paul, Sudip, et al, 2015).

## RESULTS & DISCUSSION

### Physico-chemical characterization

The physiochemical properties of *Aspergillus fumigates* 14-alpha sterol demethylase protein were assessed by ProtParam tool. The 14-alpha sterol demethylase was predicted to have molecular weight of 58930.8 Daltons and the theoretical isoelectric point (pI) of 7.64 indicating that the protein is positively charged. The instability index of the protein was computed to be 43.95, classified this protein as unstable. The negative Grand average of hydropathicity (GRAVY) index of -0.125 is indicative of a hydrophilic and soluble protein. (Kyte, J. and Doolittle, R.F., 1982).The most abundant amino acid residue was found to be Leucine (50), followed by Serine (38) and Valine (37). The lowest was found as Tryptophan. The sequence had 56 negatively charged residues (Aspartic acid + Glutamic acid) and 57 positively charged residues (Arginine + Lysine). The molecular formula, amino acid composition, atomic composition & total number of atoms of the protein are shown below.

**Figure 1:** Atomic composition of Aspergillus fumigates 14-alpha sterol demethylase assessed by ProtParam tool

**Atomic composition:**

| | | |
|---|---|---|
| Carbon | C | 2675 |
| Hydrogen | H | 4153 |
| Nitrogen | N | 703 |
| Oxygen | O | 758 |
| Sulfur | S | 20 |

**Formula:** $C_{2675}H_{4153}N_{703}O_{758}S_{20}$

**Total number of atoms:** 8309

### Aliphatic index

The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermostability of globular proteins (Ikai, A.J., 1980). The Aliphatic index of *Aspergillus fumigates* 14-alpha sterol demethylase is 89.48

**Figure 2:** Amino acid composition of Aspergillus fumigates 14-alpha sterol demethylase assessed by ProtParam tool

**Number of amino acids:** 524

**Molecular weight:** 58930.8

**Theoretical pI:** 7.64

**Amino acid composition:**

| | | |
|---|---|---|
| Ala (A) | 34 | 6.5% |
| Arg (R) | 25 | 4.8% |
| Asn (N) | 19 | 3.6% |
| Asp (D) | 28 | 5.3% |
| Cys (C) | 8 | 1.5% |
| Gln (Q) | 14 | 2.7% |
| Glu (E) | 28 | 5.3% |
| Gly (G) | 33 | 6.3% |
| His (H) | 16 | 3.1% |
| Ile (I) | 34 | 6.5% |
| Leu (L) | 50 | 9.5% |
| Lys (K) | 32 | 6.1% |
| Met (M) | 12 | 2.3% |
| Phe (F) | 28 | 5.3% |
| Pro (P) | 31 | 5.9% |
| Ser (S) | 38 | 7.3% |
| Thr (T) | 30 | 5.7% |
| Trp (W) | 7 | 1.3% |
| Tyr (Y) | 20 | 3.8% |
| Val (V) | 37 | 7.1% |
| Pyl (O) | 0 | 0.0% |
| Sec (U) | 0 | 0.0% |

**Secondary structure prediction**

A newly method known as self-optimized prediction method (SOPM) has been used in the prediction of the secondary structure of *Aspergillus fumigates* 14-alpha sterol demethylase.

**SOPMA**

| | |
|---|---|
| **Alpha helix (Hh)** | **210 is 40.08%** |
| **310 helix (Gg)** | **0 is 0.00%** |
| **Pi helix (Ii)** | **0 is 0.00%** |
| **Beta bridge (Bb) :** | **0 is 0.00%** |
| **Extended strand (Ee) :** | **91 is 17.37%** |
| **Beta turn (Tt) :** | **51 is 9.73%** |
| **Bend region (Ss) :** | **0 is 0.00%** |
| **Random coil (Cc) :** | **172 is 32.82%** |
| **Ambiguous states (?) :** | **0 is 0.00%** |
| **Other states :** | **0 is 0.00%** |

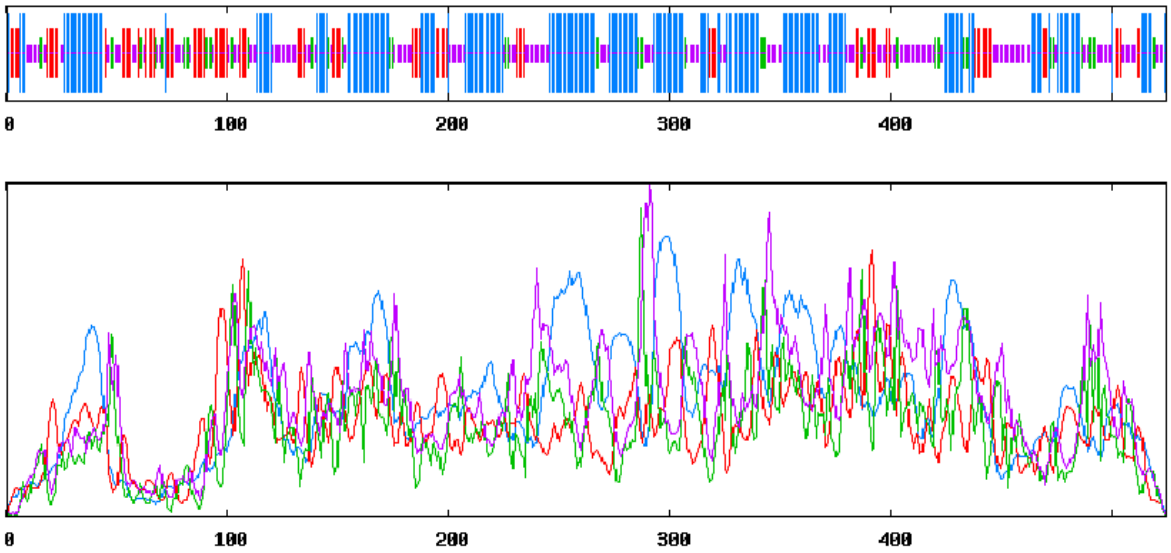
**Figure 3:** The Box representing the calculated secondary structure elements of Aspergillus fumigates 14-alpha sterol demethylase by SOPMA.

```
    10        20        30        40        50        60        70
     |         |         |         |         |         |         |
MGLIAFILDGICKHCSTQSTWVLVGIGLLSILAVSVIINVLQQLLFKNPHEPPVVFHWFPFIGSTISYGI
hhheeeehhhccccccttceeeeeecchhhhhhhhhhhhhhhhhhecttccceeeeecccettceeeetc
DPYKFFFDCRAKYGDIFTFILLGKKTTVYLGTKGNDFILNGKLRDVCAEEVYSPLTTPVFGRHVVYDCPN
ctheeeecccctttceeeeeetttceeeeeecttcceeeetcccchhhhhhhccccccccccceeeecctt
AKLMEQKKFVKYGLTSDALRSYVPLITDEVESFVKNSPAFQGHKGVFDVCKTIAEITIYTASRSLQGKEV
chhhhhtceeeetcchhhhhhhhhhhhhhhhhhhttcccccccceeeehhhhhhheeeeehccccccchh
RSKFDSTFAELYHNLDMGFAPINFMLPWAPLPHNRKRDAAQRKLTETYMEIIKARRQAGSKKDSEDMVWN
hhhhhhhhhhhhhhhcttcccceeeecccccccccccchhhhhhhhhhhhhhhhhhhhhhttcccchhhhhhh
LMSCVYKNGTPVPDEEIAHMMIALLMAGQHSSSSTASWIVLRLATRPDIMEELYQEQIRVLGSDLPPLTY
hhhhhhtttccccchhhhhhhhhhhhhhtcccccchhhheeeehcccchhhhhhhhhhhhhhhtttcccccc
DNLQKLDLHAKVIKETLRLHAPIHSIIRAVKNPMAVDGTSYVIPTSHNVLSSPGVTARSEEHFPNPLEWN
chhhhhhhhhhhhhhhhhcccchhhhhhhhcccceettceeeeecccceecctteccccccccccccccc
PHRWDENIAASAEDDEKVDYGYGLVSKGTNSPYLPFGAGRHRCIGEQFAYLQLGTITAVLVRLFRFRNLP
ttcchhhhhhhhhtthhheeeeeeeecccccccccccccccchhhhheeehttchhhhhhhhhhhtcct
GVDGIPDTDYSSLFSKPLGRSFVEFEKRESATKA
tttcccccccheeecccccceehhhhhttccch
```

**Figure 4:** Secondary structure of Aspergillus fumigates 14-alpha sterol demethylase predicted by using SOPMA C= Random coil, H= Helix, E= extended strand

```
Parameters :
   Window width         :  17
   Similarity threshold :   8
   Number of states     :   4
```

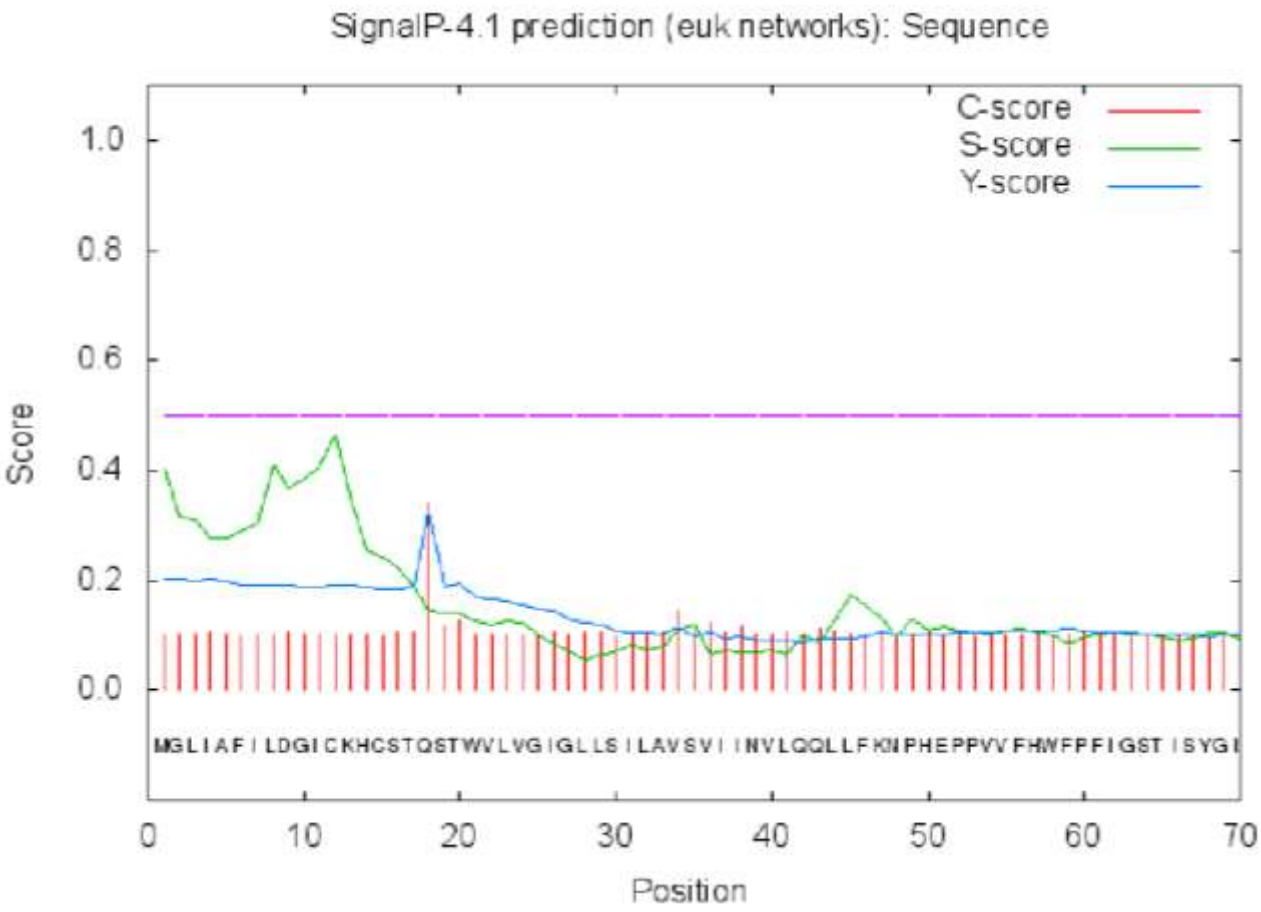
**Figure 5:** Predicted secondary structure of Aspergillus fumigates 14-alpha sterol demethylase. Here, helix is indicated by blue, while extended strands and beta turns are indicated by red and green, respectively.




he protein Sequence length was 524 Amino acid whose Alpha helix (Hh) accounts 210 amino acids of about 40.08%. The extended strand (Ee) had 91 amino acids accounting 17.37, Beta turn (Tt) made up of 51 amino acids making up 9.73% and random coil (Cc) made up of 172 amino acids accounting 32.82%.There was no $3_{10}$ helix (Gg), Pi helix (Ii), Beta bridge (Bb), Ambigous states, Bend region (Ss) and other states. The parameters were window width of 17 with a similarity threshold 8 and the number of states is 4 (Geourjon and Deleage 1995).

**Signal peptide prediction**

**Figure 6:** Signal peptide prediction by SignalP 4.1




Prediction of signalPNN using the euk networks of the SignalP 4.1 software (http://www.cbs.dtu.dk/services/SignalP/) The most likely cleavage site between the positions 17 and 18 is CST-QS.

**Subcellular localization prediction**

To Predict a subcellular localization of any unknown proteins may provide the clues about their cellular functions. This clues can be exploited in understanding the role of protein in disease mechanism and to develop drugs [Zhang R, et al 2004 ]. The subcellular localization of 14-alpha sterol demethylase Cyp51B was predicted to be a Plasma membrane protein. It was analyzed by CELLO and further authenticated by PredictProtein severs.( Paul, Sudip, et al., 2015).

**(A)**

CELLO Prediction:

Analysis Report:

| SVM | LOCALIZATION | RELIABILITY |
|---|---|---|
| Amino Acid Comp. | PlasmaMembrane | 0.626 |
| N-peptide Comp. | Peroxisomal | 0.431 |
| Partitioned seq. Comp. | PlasmaMembrane | 0.887 |
| Physico-chemical Comp. | PlasmaMembrane | 0.337 |
| Neighboring seq. Comp. | PlasmaMembrane | 0.473 |

**(B)**

**Sequence length: 524 AA**

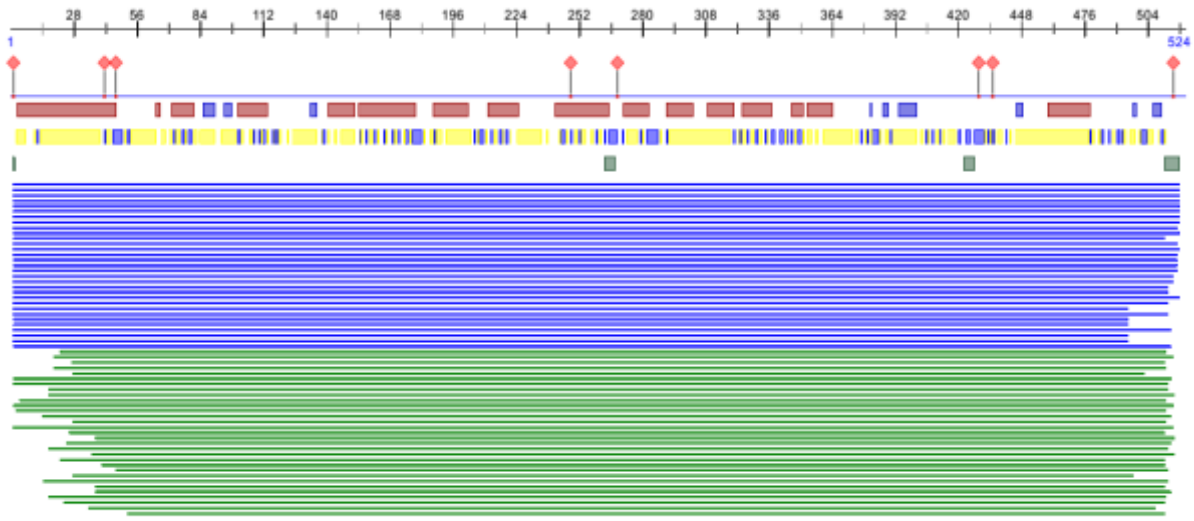
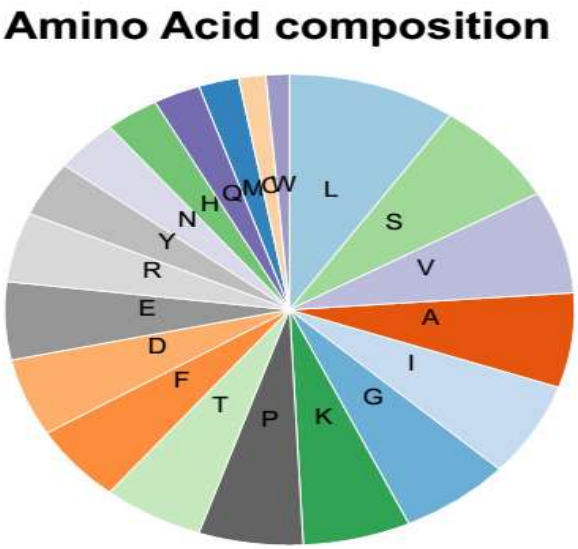



**Figure 7**: SeqID: gi|70987441|ref|XP_749134.1| 14-alpha sterol demethylase Cyp51B [Aspergillus fumigatus Af293] Subcellular localization prediction (A) By CELLO prediction (B) By PredictProtein server




The imidazole antifungals (e.g. miconazole, fluconazole and econazole) are heterocyclic synthetic compounds which inhibited the enzyme lanosterol 14 α-demethylase a necessary enzyme which convert lanosterol to ergosterol. These antifungal drugs blocks the demethylation of the C-14 of lanosterol resulting in depletion of ergosterol in fungal membrane leading to disruption of the fungal membrane structure and other essential functions of resulting in blockage of fungal growth (Diaz Guerra et al., 2003).

The present study was conducted to Physico-chemical characterization, Secondary structure prediction, Signal peptide prediction and Subcellular localization prediction of lanosterol 14 α-demethylase from *Aspergillus fumigates* which will be noteworthy for the control and designing an inhibitor against this deadly disease.

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