

## Bioinformatics analysis and modelling of mycotoxin patulin induced proteins

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### Abstract

A comparative in silico characterization of the patulin induced proteins has been carried out to analyze their physico-chemical, secondary structural and functional properties. The amino acid composition of patulin induced proteins obtained from biological databases. The composition of leucine, alanine, glycine and proline was high while low concentrations of glutamic acid and histidine residues were seen when compared to other aminoacids. The number of negative and positively charges are comparatively similar. pI value of Hyp was the highest when compared to the other two patulin induced proteins. The instability index of all the proteins was more than 40 showing that all of them are unstable. Aliphatic index shows the “relative volume of protein occupied by aliphatic side chains” which was found to be within a range of 65 to 100. Flr1P is transmembrane in nature while the other two are soluble proteins.

**Keywords:** Patulin, insilico, physico chemical properties, secondary structure

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Patulin was first isolated by Birkinshaw *et al.* [1] in 1943 from *Penicillium griseofulvum* and *Penicillium expansum*. European regulation 1425/3003 and US Food and Drug Administration (FDA) limits patulin to 50 µg/L for fruit juices. Medical problems associated with patulin exposure include neurological and gastrointestinal diseases. Most of the toxicological studies have shown the immunotoxic, genotoxic and intestinal effects of the toxin [2]. The intestinal epithelia cells are targets for these toxins [3,4]. The occurrence of patulin has been reported in apples from many countries like New Zealand, Austria, Italy, Belgium [5], Portugal [6], Canada, England, United States, Australia [7] and South Africa [8]. The role of these proteins which are induced by the toxin are unknown. Patulin induced proteins namely Flr1p, Frm2p, Hyp have been studied and their structural and compositional analysis has been done in this

study using various computational tools and servers. Many factors affect the expression of patulin like nitrogen source, metal ions etc. Patulin is produced by *Aspergillus*, *Paecilomyces*, *Penicillium* and *Byssoschlamys*. Around 15 genes involved in patulin biosynthesis have been reported in the *A. clavatus* genome [9]. In the present study, a computational analysis of patulin induced proteins has been done and the results are discussed

### Materials and Methods

UniProtKB/Swiss-Prot was used to retrieve the complete sequences of the patulin induced proteins. The computation of various physical and chemical parameters of the patulin induced proteins (aminoacids, molecular weights, pI, negative and positive charged residues, extinction coefficient, instability index, aliphatic index and GRAVY) was

**Table 1: Sequences of patulin induced proteins retrieved from Protein databases**

| # | Sequences  | Name of Protein       |
|---|--|-----------------------|
| 1 | MVYTSTYRHTIVVDLLEYLGIVSNLETLQSAREDETRKPENIDKKECKPDYDIECGPNRSCSESTDSGSGQIEKN<br>DPERVDWNGPSPDPENPQNWPPLKKSLLVVFQIMLLTCTVTYMGSSITPGQEYIQEEFHVGHVVVATLNLISLYVLGYGL<br>GPIIFSPLETA RYGRLNLY<br>MVTLFFFMIFQVGCATVHNIGGLIVMRFISGILCSPSLATGGGTVADIISPEMVPPLVLMWSAGAVAAP<br>VLAPLLGAA MVDAKN WRFIFWLLMWLSAATFILLAFFPETQHHNILYRRALKLRKETGDDRYYTE<br>QDKLDREVD ARTFLINTLY RPLKMIHK EPAILAF<br>DLYAVAYGCFYLFEEAFPIVFGIYHFSLSVEVGLAYMGFCVGCVLAYGLFGLNMRIVPRFRNGTFTPEAF<br>LIVAMC V CWCLPL SLFLFGWTARVHWILPVISEVFFLAVFNIFQATFAYLATCYPKYVASVFA GNGFCRASFACA<br>FPLFGRA MYDNLAT KNYPVAW GSSLVGFLLGLAIIPFILYKYGPSLRTRSSYTEE<br>MSPTGNYLNAITNRRITNYNLKPELPQGVGLDDVKRTVHVILKNTPTAFNSQVNRAVIIVGDTHKRIWD AVASAMP<br>TAEAKKRPE<br>SCRDEAYGSVIFFTDEGPTTEKLRDFPALAAAFPTCAAHTTGAVQIQSWTALLELLGLGANLQHYNDYVKSALPQD<br>VPIAWTVQ SQLVFGVPTALPEEKTFINNVVYH | Fir1p                 |
| 2 | MYIPKHFESMELSRYLKSKKPPPLGTLFSSKASRQGFHWRTSSNKDDPDFGMCA SHIPFVFEFDNGEHKLIHLA<br>RKNKQVEMLERVQKCLVVFQSV<br>DSYISPAWFPMKKKTTHKFVPTWDFAVHVYGTPIRIIRDDKDWLNLMLSTLTDQ EEEKRPEGENVRSKVERF   | Frm2p                 |
| 3 |  | Hyp<br>CENPK1137D_933 |

**Table 2: Amino acid composition of Patulin induced proteins**

|            | Flr1p | Frm2p | Hyp  |
|------------|-------|-------|------|
| <b>Ala</b> | 7.7   | 10.9  | 4.1  |
| <b>Arg</b> | 4.4   | 4.1   | 5.9  |
| <b>Asn</b> | 3.5   | 6.2   | 3.0  |
| <b>Asp</b> | 3.5   | 4.7   | 5.9  |
| <b>Cys</b> | 2.7   | 1.0   | 1.2  |
| <b>Gln</b> | 1.8   | 4.7   | 3.0  |
| <b>Glu</b> | 4.7   | 4.7   | 7.1  |
| <b>Gly</b> | 6.9   | 5.2   | 4.1  |
| <b>His</b> | 1.5   | 2.6   | 3.6  |
| <b>Ilu</b> | 6.6   | 5.7   | 4.1  |
| <b>Leu</b> | 11.5  | 7.8   | 6.5  |
| <b>Lys</b> | 2.9   | 4.7   | 10.1 |
| <b>Met</b> | 2.7   | 1.0   | 3.6  |
| <b>Phe</b> | 7.7   | 3.6   | 7.7  |
| <b>Pro</b> | 5.3   | 6.7   | 5.9  |
| <b>Ser</b> | 6.2   | 4.1   | 8.3  |
| <b>Thr</b> | 5.7   | 8.8   | 4.1  |
| <b>Trp</b> | 1.8   | 1.6   | 2.4  |
| <b>Tyr</b> | 5.3   | 3.1   | 2.4  |
| <b>Val</b> | 7.7   | 8.8   | 7.1  |

**Table 3: Physico-chemical parameters of Patulin induced proteins**

| Protein | No. of A.A. | M.W (Da) | pI   | "-" charged residues | "+" charged residues | Extinction coefficient | Instability index | Aliphatic index | GRAVY  |
|---------|-------------|----------|------|----------------------|----------------------|------------------------|-------------------|-----------------|--------|
| Flr1p   | 548         | 61628.9  | 5.96 | 45                   | 40                   | 99085                  | 40.52             | 100.35          | 0.410  |
| Frm2p   | 193         | 21232.1  | 6.51 | 18                   | 17                   | 25565                  | 49.05             | 88.96           | -0.172 |
| Hyp     | 169         | 19818.8  | 9.18 | 22                   | 27                   | 28085                  | 41.85             | 66.27           | -0.575 |

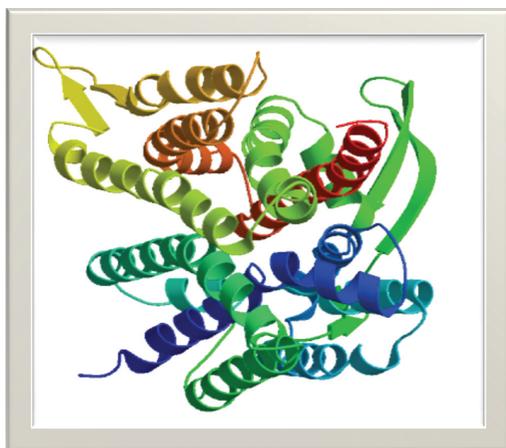
**Table 4: Secondary Structural Features of proteins using SOPMA**

| Name of the protein | $\alpha$ helix | 310 helix | Pi helix | $\beta$ bridge | Extended strand | $\beta$ turn | Bend region | Random coil | Ambiguous | Other |
|---------------------|----------------|-----------|----------|----------------|-----------------|--------------|-------------|-------------|-----------|-------|
| Flr1p               | 42.34%         | 0         | 0        | 0              | 17.70           | 3.47         | 0           | 36.50       | 0         | 0     |
| Frm2p               | 49.22          | 0         | 0        | 0              | 11.92           | 5.70         | 0           | 33.16       | 0         | 0     |
| Hyp                 | 23.67          | 0         | 0        | 0              | 20.12           | 4.14         | 0           | 52.7        | 0         | 0     |

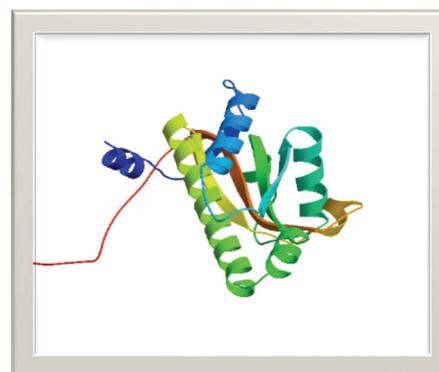
**Table 5: Transmembrane regions of patulin induced proteins**

| Name of the protein | Nature of protein | N – terminal | Transmembrane region    | C terminal | Type      | Length |
|---------------------|-------------------|--------------|-------------------------|------------|-----------|--------|
| <b>Flr1p</b>        | Transmembrane     | 1            | MVYTSTYRHTIVVDLLEYLGIVS | 23         | Secondary | 23     |
|                     |                   | 103          | SLVVFQIMLLTCVTYMGSSIYTP | 125        | Primary   | 23     |
|                     |                   | 138          | HVVATLNLSLYVLGYGLGPIIFS | 160        | Secondary | 23     |
|                     |                   | 180          | FFMIFQVGCATVHNIGGLIVMRF | 202        | Primary   | 23     |
|                     |                   | 226          | EMVPLVLGMWSAGAVAAPVLAPL | 248        | Primary   | 23     |
|                     |                   | 261          | FIFWLLMWSAATFILLAFFFP   | 282        | Primary   | 22     |
|                     |                   | 341          | LAFDLYIAVAYGCFYLFPEAFPI | 363        | Primary   | 23     |
|                     |                   | 378          | LAYMGFCVGCVLAYGLFGILNMR | 400        | Primary   | 23     |
|                     |                   | 413          | PEAFLIVAMCVCWCLPLSLFLFG | 435        | Primary   | 23     |
|                     |                   | 445          | PVISEVFFVLAVFNIFQATFAYL | 467        | Primary   | 23     |
|                     |                   | 512          | AWGSSLVGFLTGLAIPFILYK   | 534        | Secondary | 23     |
| <b>Frm2p</b>        | Soluble           | —            | —                       | —          | —         | —      |
| <b>Hyp</b>          | Soluble           | —            | —                       | —          | —         | —      |

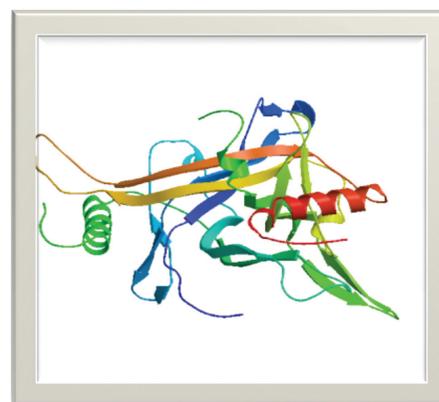
done using ExPASy’s ProtParam tool. ExPASy’s ProtScale tool was used to analyse hydrophobicity and transmembrane tendency [10]. SOPMA tool server was used to characterize the secondary structural features [11]. The analysis of the patulin induced proteins motifs was done with the help of Motif Scan tool [12]. The SOSUI server prediction yielded the transmembrane regions of the patulin induced proteins [13]. Protein modelling was done using swiss model software



**Fig. 1: Model generated of the Flr1p protein by swiss model software**



**Fig. 2: Model generated of the Frm2p protein by swiss model software**



**Fig. 3: Model generated of the Hyp protein by swiss model software**

## Results and Discussion

Increase in the levels of nutrient nitrogen in the medium determines the expression of patulin pathway, Grootwassink and Gaucher [14]. This type of down-regulation is also observed in sterigmatocystin and ochratoxin A [15, 16]. Carbon source in the medium is not influencing the production of the toxin while metal ions such as Manganese are essential requirement for patulin biosynthesis [17, 18]. The exact effect of many of the patulin gene cluster remains unclear and needs to be explored [19-21]. The levels of patulin are high in all stages of the apple product processes,. A better understanding of patulin proteins would help us to inhibit the synthesis or target these proteins or identify the levels of patulin production. Patulin induced proteins obtained from database are presented in Table 1. Amino acid composition of patulin induced proteins obtained from biological databases (Table 2). The composition of leucine, alanine, glycine and proline was high while low concentrations of glutamic acid and histidine residues were seen when compared to other aminoacids. Negative and positively charged aminoacids numbers are comparatively similar (Table 3). Molecular weight of Flr1p was the highest while the other two showed relatively less molecular weights. pI value of Hyp was the highest when compared to the other two patulin induced proteins. The instability index of all the proteins was more than 40 showing that all of them are unstable. Aliphatic index was found to be within a range of 65 to 100. From Table 4, dominance of  $\alpha$ -helices and random coils was observed from the secondary structural analysis of the proteins. SOSUI server analysis (Table 5) has shown that Flr1P is transmembrane in nature while the other two are soluble proteins. Modelling of the proteins was done using Swiss model software (figure 1,2,3). In this study, physico-chemical, secondary structural and functional analysis of the large human patulin induced proteins family was carried out. These Insilico findings can be used for working on patulin induced proteomic properties in solution.

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