"Studies On Myrmicitoxin From The Ants On Aphids Using Insilico Analysis"

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Abstract

Acyrthosiphon pisum, also known as the pea aphid, is an insect responsible for hundreds of millions of dollars of crop damage every year. Myrmicitoxin so far identified are named as a potent peptide precursors from the venoms of ants are promising molecules for aphid control using various bioinformatics tools. The myrmicitoxin includes other toxins that are directed against various organism was studied. Six toxins were identified for myrmicitoxins and they are one M-myrmicitoxin(01)-Tb1a, four U-myrmicitoxin(01)-Tb2a,Tb3a,Tb4a,Tb5a and one U1-myrmicitoxin(01)-Mr1a. But for this study only myrmicitoxin(01)-Mr1a act as an attractive candidates for the development of novel insecticidal potential against pea aphids, Acyrthosiphon pisum (*Heep, J* et al.,2019).

Keywords: Acyrthosiphon pisum, Myrmicitoxin, aphids, M. rubida venom peptidome

Introduction

"Venomics" study was initiated by peptidomics, more than 2,800 venom peptides were deposited in protein database, most of which were fragments of 37 full-length peptide precursors called myrmicitoxins. To perform the in silico analysis of molecular diversity of the venom peptidome for the myrmicine ant Tetramorium bicarinatum, the study was investigated using various proteomics tool. The toxins that are taken for studies one M-myrmicitoxin(01)-Tb1a, four U-myrmicitoxin(01)-Tb2a,Tb3a,Tb4a,Tb5a and one U1-myrmicitoxin(01)-Mr1a, which are directed against insects and ants. The sequence is downloaded from Swissprot a biological database of protein sequences. The structure analysis includes the prediction of primary, secondary and post translational modification study. The functional part includes the identification of domain hits, disordered region and the motif identification in these toxins. And finally a phylogenetic tree is constructed for these toxins to study their phylogenetic relation. Various proteomics tool such as protparam, coils, GOR and signal are used in the structure analysis. And for functional analysis tools like SMART, MotiFinder, DisEMBL are used. The multiple sequence alignment is carried out using CLustalW, a server and the phylogenetic tree is constructed using BLOSUM62 that comes along with ClustalW server. The tree is constructed based on the average distance algorithm.

Materials Required:

The study is aimed at the characterization of the structural and functional properties of these proteins. Initially the sequences were retrieved from the swissprot knowledgebase, a protein sequence database. The length of sequences seems to contain below 80 aminoacid residues. These amino acid sequences are converted to FASTA format a valid format used in proteomic analysis.

The structure analysis include the primary, secondary and tertiary. The primary structure analysis of these toxins show the physico chemical properties such as molecular weight, theoretical PI, the aminoacid composition, the total number of positively or negatively charged residues, and other parameters. These calculations are tabulated in Table. No. 1

The secondary structure analysis is carried out using GOR and the result obtained shows the presence of the alpha helix (red in color), beta sheets (blue in color) and turns (violet in color) regions in a graphical format for these toxins are shown in the Fig.No.1

Finally in tertiary structure analysis the post translational modification of these toxins was carried out by using the tool SignalP which shows the possible occurrence of signal peptide and the result comprises the cleavage site predicted and the mean of their prediction. The graphical representation of the result is shown in Fig.No.2. Comparing other strong toxins, the U-myrmicitoxin-Mrla shows a constant hit for signal peptides.

After the structural analysis, the functional analysis of the protein domain Fig. No.3 carried out by using the tool SMART. The result shows that presence of transmembrane region and low complexity region inM-myrmicitoxin(01)-Tb1a, U-myrmicitoxin(01)-Tb2a, U-myrmicitoxin(01)-Tb3a, U-myrmicitoxin(01)-Tb4a and U-myrmicitoxin(01)-Tb5a .

The disordered regions found in these were identified using DisEMBL which shows the disordered hot loops highlighted by red color in the sequence, disordered loops or coils in Blue color and finally the re-mark-465 in green color. This is also represented as graph from Fig.No.4.

Finally phylogenetic analysis predicts the phylogeny for protein analysis. The result shown in the Fig.No.5 shows that the U-myrmicitoxin(01)-Tb5a and U-myrmicitoxin(01)-Tb4a must have originated from the same ancestor and others shows conserved grouping among U-myrmicitoxin(01)-Tb3a, U-myrmicitoxin(01)-Tb2a, M-yrmicitoxin(01)- Tb1a. Among all, the U1-myrmicitoxin-Mr1a was found to be an outgroup divergentwith distance 5.2606425.

Result and Discussion

PRIMARY STRUCTURE USING PROTPARAM

Table No.1

Protein name	Formula	No. of Aa.	No.of atoms	Mol.wt	pI	Instability index	Aliphatic index	Positively charged residue	Negatively charged residue	GRAVY
M-myrmicitoxin (01)-Tb1a	C372H602N92O105S4	79	1175	8171.67	5.59	-6.17	111.52	9	10	0.565
U-myrmicitoxin (01)-Tb2a	C276H443N67O90S3	60	879	6236.13	4.07	20.89	106.33	4	10	0.537
U1-myrmicitoxin-Mr1a	C50H87N11O16	10	164	1098.30	4.37	19.77	166.00	1	2	0.440
U-myrmicitoxin (01)-Tb3a	C266H423N73O76S6	56	844	6052.09	7.80	37.84	82.14	6	5	0.239
U-myrmicitoxin (01)-Tb4a	C260H419N77O72S6	54	834	5968.02	7.88	17.97	92.41	5	4	0.241
U-myrmicitoxin (01)-Tb5a	C265H403N67O77S7	54	819	5983.94	4.16	18.14	86.85	1	6	0.363

SECONDARY STRUCTURE PREDICTION

GOR

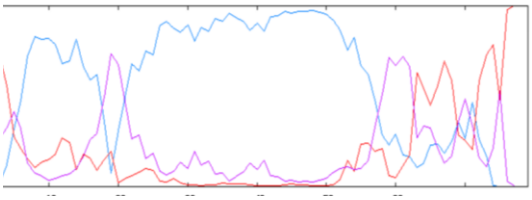

Fig No.1: Graphs showing the secondary structure of the myrmicitoxin

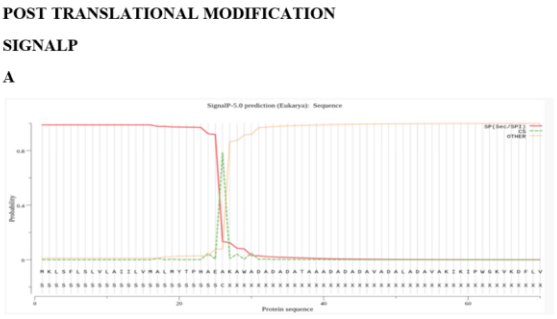

Fig No.2: Graphs showing the signal peptides occurred in myrmicitoxin

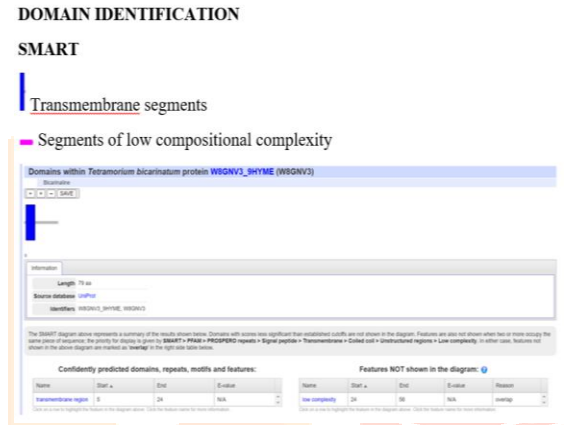

Fig No.3 SMART detects the specific domain region for M-myrmicitoxin(01)-Tb1a

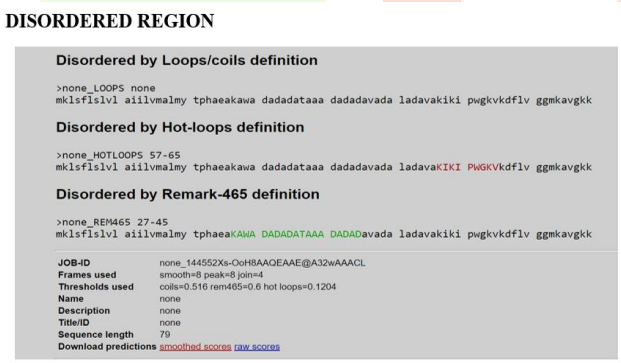

Fig No.4: DisEMBL predicts the disordered region of the peptide-binding sites, M-myrmicitoxin(01)-Tb1a

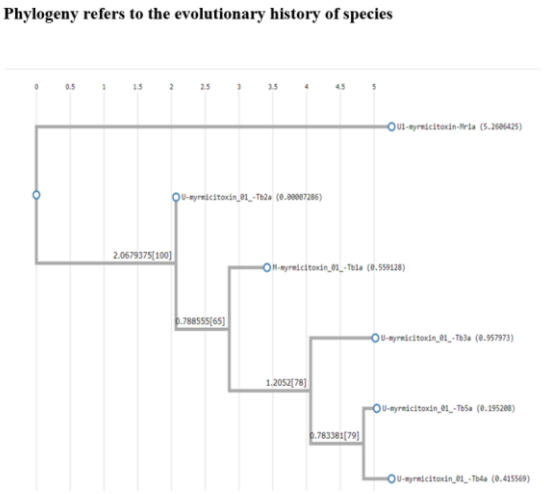

Fig No.5: Phylogeny relationship among species results in higher the bootstrap value, higher the confidence level of the clade in the phylogenetic tree

Conclusion

Myrmicitoxin, a potent peptide precursors from the venoms of ants are promising molecules for aphid control is analyzed using various bioinformatics tools. The myrmicitoxin includes other toxins that are directed against various organism was studied. The structure and functional analysis reveals various information about these toxin proteins. From this analysis one can infer that these toxin proteins have low complexity transmembrane region. Also it shows that these toxin proteins identified the signal peptides. Further this myrmicitoxin can be studied for its application in medicine field. Therefore, we explored the M. rubida venom peptidome identified a novel reduced aphid survival and reproduction. The remarkable insecticidal activity of M. rubida venom suggests it may be a promising source of additional bio-insecticide leads.

Acknowledgement

The author thank the Management and the Principal Dr. G. Geetha of Thiru.Vi.Ka.Govt Arts College-610 003, Thiruvarur District, Tamilnadu, for providing necessary facilities.

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