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Analysis of Ribosome Inactivating Protein (RIP): A Bioinformatics Approach

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ABSTRACT

In spite of the medical advances in recent years, the world is in need of different sources to encounter certain health issues. Ribosome Inactivating Proteins (RIPs) were found to be one among them. In order to get easy access about RIPs, there is a need to analyse RIPs towards constructing a database on RIPs. Also, multiple sequence alignment was done towards screening for homologues of significant RIPs from rare sources against RIPs from easily available sources in terms of similarity. Protein sequences were retrieved from SWISS-PROT and are further analysed using pair wise and multiple sequence alignment. Analysis shows that, 151 RIPs have been characterized to date. Amongst them, there are 87 type I, 37 type II, 1 type III and 25 unknown RIPs. The sequence length information of various RIPs about the availability of full or partial sequence was also found. The multiple sequence alignment of 37 type I RIP using the online server Multalin, indicates the presence of 20 conserved residues. Pairwise alignment and multiple sequence alignment of certain selected RIPs in two groups namely Group I and Group II were carried out and the consensus level was found to be 98%, 98% and 90% respectively.

Keywords: Ribosome Inactivating Proteins (RIPs), Database, Screening, Pair wise alignment, Multiple Sequence Alignment.

1 INTRODUCTION

A biological database is a large, organized body of persistent data, usually associated with computerized software designed to update, query, and retrieve components of the data stored within the system [1]. A simple database might be a single file containing many records, each of which includes the same set of information. For researchers to benefit from the data stored in a database, two additional requirements must be met: 1. Easy access to information; and 2. A method for extracting only that information needed to answer a specific biological question.

Proteins with selective toxicity have been investigated for use in ways as varied as murder weapons by mystery writers [2] and espionage agents [3], [4] to transgenic plant protection by biologists [5], [6], [7] "silver bullet" therapies by cancer researchers [8], [9], [10], [11], [12], [13] and biological weaponry by military groups [14]. One class of such proteins, ribosome-inactivating proteins (RIPs), are found in genera throughout the plant kingdom as well as in certain fungi and bacteria. Ribosome-inactivating proteins (RIPs) are a group of proteins that share the property of damaging ribosomes in an irreversible manner, acting catalytically, i.e. enzymatically [15], [16], [17].

RIP's have been proven to play a vital role in development of novel drugs as these are precursors for several synthetic drugs many of them act as lead compounds for several synthetic ones. The synthetic drugs are mostly of chemical origin and therefore are less preferred over those of natural origin as these are with negligible side effects and are also cost effective .There are several drugs of natural origin for instance anticancer drugs, Vincristine and Vinblastine complexes obtained from the Rosey Periwinkle (*Catharanthus rosea*) and many more drugs of natural origin are being effectively used for the treatment of many dreadful diseases [18].

The Ribosome Inactivating Proteins (RIP's) inactivates the eukaryotic ribosomes these are generally the plant proteins which depurinates the rRNA and therefore are the inhibitors of protein synthesis. The RIP's shows several pharmacological properties, which mainly includes Anti-HIV, Antitumor and Abortifacient properties [19]. Ribosome Inactivating Proteins (RIP's) induces apoptosis by decreasing the action of anti-apoptotic factors they have antiviral and anti-parasitic properties and have proved to be a very effective drug against AIDS by acting directly on HIV infected cells by depurinating the RNA. The RIP's are better cure for certain allergies but are also having allergenic properties as they are raw eaten in the form of vegetables. RIP's as immunotoxins are potentially used to treat the tumor cells. The abortifacient property of RIP's has been reported in

various plants species as they inhibit the protein synthesis [20]. Since the drugs of natural origin are more efficient and cost effective over the synthetic drugs. Therefore new drug alternatives from plants should be identified and designed in order to obtain drugs with negligible side effects[21]. In this paper, phyto-protein mainly the ribosome inactivating proteins (RIPs) are taken for analysis.

Since RIPs exhibit antiviral, antifungal, immunological activities the detailed knowledge of their sequence and structural information will be useful in therapeutic applications. However, there is no database to provide such information. The aim of the present study is thus to analyse RIPs of various types database creation. It also includes the screening for homologues of significant RIPs from easily available source sharing high percentage of similarity with RIPs from rarely available source for therapeutic applications.

2 MATERIALS AND METHODS

2.1. Retrieval of RIP sequences from the Swiss-Prot, Protein Database

The sequences of RIPs were searched and retrieved in the FASTA format from the SWISS-PROT, protein database and is tabulated.

2.2. The similarity search done across Ribosome inactivating protein (RIP) sequences

The alignments of RIPs with two isoforms were generated by pairwise alignment using Smith-Waterman algorithm and the similarity was noted towards studying the relationship between sequences [22]. The sequences showing more than 80% similarity were considered.

2.3. Performing the Multiple Sequence Alignment for the similar RIP sequences

Multiple sequence alignments were carried out using the online server Multalin to study the similarity between sequences [23] and to find the conserved regions among the sequences.

3 RESULTS AND DISCUSSIONS

3.1. Retrieval of RIP sequences from the Swiss-Prot, Protein Database

RIPs are known to be produced by a wide variety of plants. The sequences were retrieved from SWISS-PROT Database. Our analysis shows that 151 RIPs have been characterized to date. Among them there are 87 type I, 37 type II, 1 type III and 25 unknown RIPs. The information of various RIPs about the total number of RIPs with full and partial sequence, partial sequence information of type I RIPs and full sequence information of type II RIPs are given in the tables 1, 2 and 3 respectively.

Table.1 Numbers of RIPs with full and partial sequence

Type	**Full sequence**	**Partial sequence**
I	42	45
II	17	20
III	1	-
Unknown	12	13

Table.2 The partial sequence information of type I RIPs

SWISS-PROT ID	**Sequence information**	**Total number of amino acids**
RIP1_SAPOF	partial	40
RIP2_DIACA	partial	60
RIP2_TRIKI	partial	45
RIP3_GELMU	Partial	55
RIP3_SAPOF	Partial	236
RIP4_SAPOF	Partial	157
RIPA_LUFCY	Partial	277
RIPK_TRIKI	Partial	16
RIPS_MOMCO	Partial	30
RIPX_CUCPE	Partial	20
RIP_CUCMO	Partial	27
RIP_LUFAC	Partial	10
RIP_SIRGR	Partial	18

P83206_GYNPE	Partial	19
Q2XXE5_ZEADI	Partial	299
Q2XXE6_ZEADI	Partial	302
Q2XXE7_ZEADI	Partial	293
Q2XXE8_ZEADI	Partial	300
Q2XXE9_ZEADI	Partial	295
Q2XXF0_ZEADI	Partial	303
Q2XXF1_ZEADI	Partial	303
Q2XXF2_ZEADI	Partial	297
Q2XXF3_ZEAMP	Partial	300
Q2XXF4_ZEAMP	Partial	300
Q2XXF5_ZEAMP	Partial	308
Q2XXF6_ZEAMP	Partial	303
Q2XXF7_ZEAMP	Partial	301
Q2XXF8_ZEAMP	Partial	302
Q2XXF9_ZEAMP	Partial	303
Q2XXG0_ZEAMP	Partial	304
Q2XXG1_ZEAMP	Partial	296
Q2XXG2_ZEAMP	Partial	300
Q2XXG3_ZEAMP	Partial	301
Q2XXG4_ZEAMP	Partial	301
Q2XXG5_ZEAMP	Partial	300
Q41176_LUFCY	Partial	285
Q84LI8_CUCMO	Partial	136
Q8S2R5_CUCMO	Partial	136
Q8GV09_GYNPE	Partial	277
Q8GV10_GYNPE	Partial	277
Q8GV11_GYNPE	Partial	277
Q8H1Y4_GYNPE	Partial	275
Q8H1Y5_GYNPE	Partial	277
Q8SAG0_BENHI	Partial	136
Q9FS39_MAIZE	Partial	301
RIPT_TRIKI	Partial	289

Table.3 Full sequence information of type I RIPs

SWISS-PROT ID	**Sequence information**	**Total number of amino acids**
RIP0_DIACA	Full	293
RIP2_SAPOF	Full	292
RIP6_SAPOF	Full	299
RIP5_SAPOF	Full	253
RIP7_SAPOF	Full	253
Q93Y66_9CARY	Full	294
RIP1_PHYAM	Full	313
Q8RYA4_PHYAM	Full	339
RIPA_PHYAM	Full	294
RIP2_PHYDI	Full	265
RIPS_PHYAM	Full	261
RIP2_PHYAM	Full	310
Q9M5K6_CHEAL	Full	279
RIPP_MIRJA	Full	278
Q8GV51_9CARY	Full	317
Q9SAQ5_AMAVI	Full	270
RIP1_BRYDI	Full	290
Q53X19_BRYDI	Full	290

RIPS-TRIKI	Full	289
RIP1_MOMCH	Full	286
RIP1_CUCFI	Full	286
RIP1_TRIAN	Full	294
RIP2_MOMBA	Full	286
RIP3_MOMCH	Full	286
RIPB_LUFCY	Full	250
RIP2_BRYDI	Full	282
004358_IRIHO	Full	300
004358_IRIHO	Full	298
RIPG_GELMU	Full	316
Q8GZN9_9ROS	Full	299
Q8GZPO_9ROS	Full	297
RIP1_HORVU	Full	280
RIP2_HORVU	Full	280
RIP3_MAIZE	Full	300
RIP9_MAIZE	Full	304
RIPX_MAIZE	Full	301
Q7ISN2_MUSAR	Full	294
Q8L5M2_MUSAR	Full	295
Q8L5M3_MUSAR	Full	294
Q8L5M4_MUSAR	Full	298
Q599X2_9CARY	Full	283
Q6VIW8_AMATR	Full	297

3.2. Pairwise alignment of RIPs, RIP1_HORVU and RIP2_HORVU

In table 4, it is noticed that, *Hordeum Vulgare* possess two isoforms. In order to study the difference between these isoforms at the sequence level, pairwise alignment was carried out using Smith-Waterman algorithm [22] and the result shows that they were 98% identical (fig. 1).

>sp|P22244|RIP1_HORVU

AAKMAKNVDKPLFTATFNVQASSADYATFIAGIRNKLRN-
PAHFSHNRPVLPPVEPNVPPSRWFHVVLKASPTSAGLT-
LAIRADNIYLEGFKSSDGTWWELTPGLIP-
GATYVGFGGTYRDLLGDTDKLT-
NVALGRQQLADAVTALHGRTKAD-
KPSGPKQQQAREAVTTLLLMVNE-
ATRFQTVSGFVAGLLHPKAVEKKSG-
KIGNEMKAQVNGWQDLSAALLKTDVKPPPGK-
SPAKFAPIEKMGVRTAVQAANTLGILLFVEVPGGLT-
VAKALELFHASGGK

>sp|P04399|RIP2_HORVU

AAKMAKNVDKPLFTATFNVQASSADYATFIAGIRNKLRN-
PAHFSHNEPVLPPVEPNVPPSRWFHVVLKASPTSAGLT-
LAIRADNIYLEGFKSSDGTWWELTPGLIP-
GATYVGFGGTYRDLLGDTDKLTNVALGRQQLE-
DAVTALHGRTKADKASGPKQQQAREAVTLLLMVNE-
ATRFQTVSGFVAGLLHPKAVEKKSG-
KIGNEMKAQVNGWQDLSAALLKTDVKPPPGK-
SPAKFTPIEKMGVRTAEQAAATLGILLFVEVPGGLT-
VAKALELFHASGGK

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RIP1_HORVU         1 RWFHVVLKASPTSAGLTLAIRADNIYLEGFKSSDGTWWELTPGLIPGATY     50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
RIP2_HORVU         1 RWFHVVLKASPTSAGLTLAIRADNIYLEGFKSSDGTWWELTPGLIPGATY     50

RIP1_HORVU        51 VGFGGTYRDLLGDTDKLTNVALGRQQLADAVTALHGRTKADKPSGPKQQQ    100
                     ||||||||||||||||||||||||||||||.|||||||||||||||.|||||||
RIP2_HORVU        51 VGFGGTYRDLLGDTDKLTNVALGRQQLEDAVTALHGRTKADKASGPKQQQ    100

RIP1_HORVU       101 AREAVTTLLLMVNEATRFQTVSGFVAGLLHPKAVEKKSGKIGNEMKAQVN    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||
RIP2_HORVU       101 AREAVTTLLLMVNEATRFQTVSGFVAGLLHPKAVEKKSGKIGNEMKAQVN    150

RIP1_HORVU       151 GWQDLSAALLKTDVKPPPGKSPAKFAPIEKMGVRTAVQAANTLGILLFVE    200
                     ||||||||||||||||||||||||||||.||||||||||.|||.|||||||||
RIP2_HORVU       151 GWQDLSAALLKTDVKPPPGKSPAKFTPIEKMGVRTAEQAAATLGILLFVE    200

RIP1_HORVU       201 VPGGLTVAKALELFHASGGK    220
                     ||||||||||||||||||||
RIP2_HORVU       201 VPGGLTVAKALELFHASGGK    220
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Fig.1 Pairwise Sequence Alignment of RIP1_HORVU, RIP2_HORVU

3.3. Multiple Sequence Alignment of RIPs

Multiple sequence alignment was performed using the online server Multialn to search for the homologues among the sequences and conserved sequences and the alignment is shown in Fig.2(a) and Fig.2(b). Analysis shows that 20 residues are conserved (indicated in letters grey in Fig.2(a) and Fig. 2(b)).

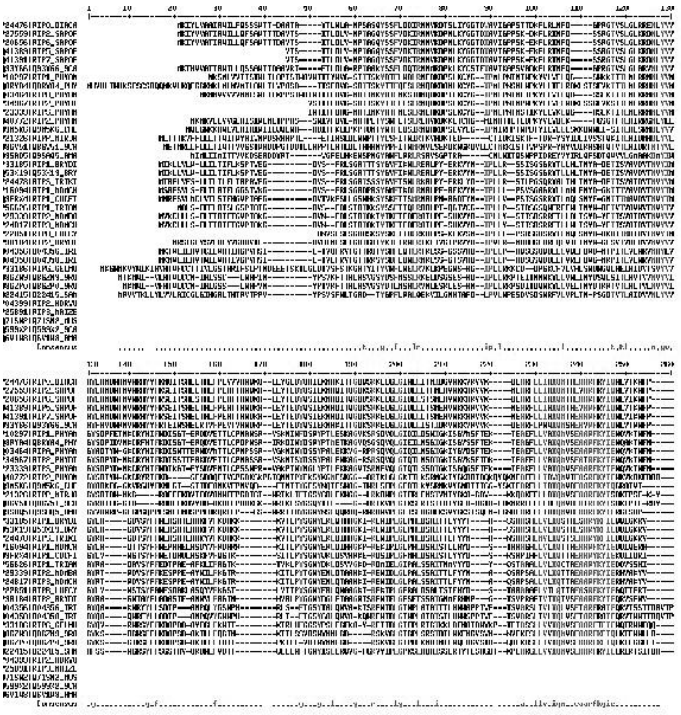

Fig.2(a) Multiple sequence alignment of 37 type I RIPs.

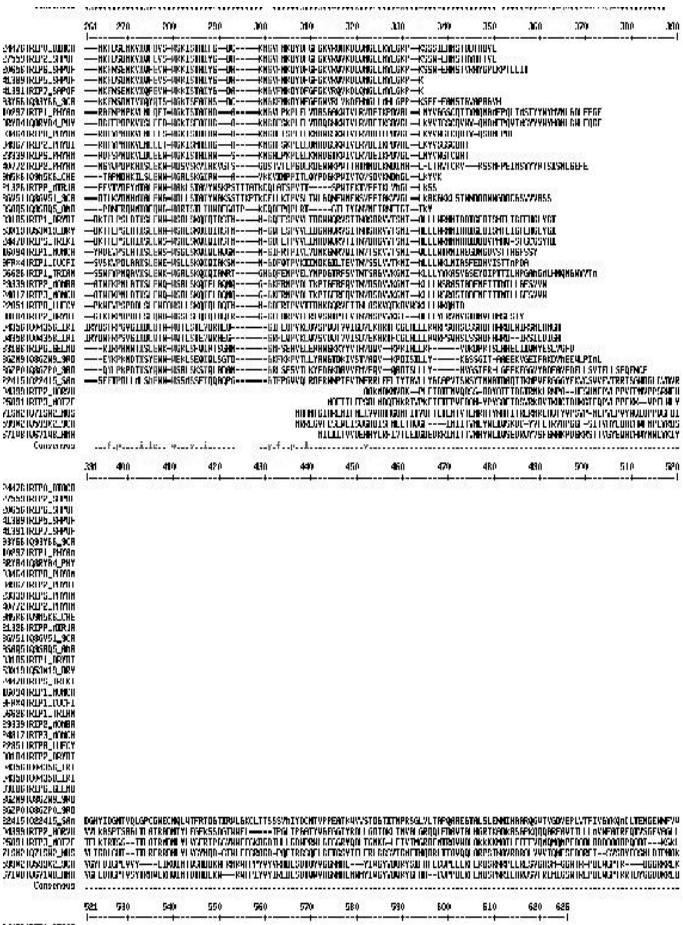

Fig.2(b) Multiple sequence alignment of 37 type I RIPs.

3.3.1. Multiple Sequence Alignment of RIP9_MAIZE, RIPX_MAIZE and RIP3_MAIZE

In table 3, it is noticed that *Zea mays* contains three isoforms. To check how these isoforms differ at the sequence level, Multiple Sequence Alignment [23] was carried out and the result shows that they are 98% identical (fig.3). Similar sequences are indicated in grey colour.

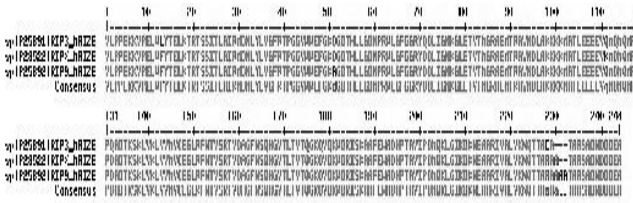

Fig.3 Multiple sequence alignment of RIP3_MAIZE, RIP9_MAIZE, RIPX_MAIZE

3.3.2. Multiple Sequence Alignment of Q71SN2_MUSAR, Q8L5M2_MUSAR, Q8L5M3_MUSAR, Q8L5M4_MUSAR

While generating the multiple sequence alignment, the consensus level was found to be 90% (fig.4). Similar sequences are indicated in grey colour.

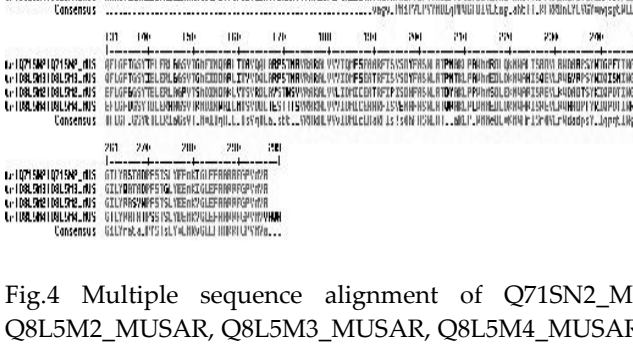

Fig.4 Multiple sequence alignment of Q71SN2_MUSAR, Q8L5M2_MUSAR, Q8L5M3_MUSAR, Q8L5M4_MUSAR.

4 CONCLUSIONS

RIPs are abundant in nature and they exhibit antiviral, antifungal and insectisidal activities. The detailed sequence and structural information of RIP will therefore be useful for therapeutic applications. However, a database on the required information about RIPs is lacking. As a preliminary step towards creating a database we have analysed the sequences RIPs. The present analysis shows that there are 87 type I RIP, 37 typeII RIP, 1 typeIII RIP and 25 unknown RIP. The sequence alignment of 37 typeI RIP shows 20 conserved

residues. Pairwise alignment of RIP1_HORVU, RIP2_HORVU has been carried out and the result shows 98% similarity. Multiple sequence alignment of RIP3_MAIZE, RIP9_MAIZE, and RIPX_MAIZE has been carried out to find the similarity and the result shows **98%** similarity. Multiple sequence alignment of Q71SN2_MUSAR, Q8LSM2_MUSAR, Q8L5M3_MUSAR, Q8L5M4_MUSAR shows **90%** similarity. The significance of the work is, similar type of RIPs were analysed using appropriate tools to select RIPs of therapeutic applications with ease.

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