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Analysis of Modules in Protein-Protein Interaction Network of Clostridium tetani

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ABSTRACT Now-a-days, Graph-based approaches are widely used for the analysis of the different PPI networks such as for Clostridium tetani. As we know that most of the real network follows the scale free power law distribution. Thus the interactions among the proteins in the network follow heterogeneity with the other proteins in the bacteria due to the specific nature and characteristics of some of the proteins, whereas the random interactions among proteins follow homogeneity. This happens due to cohesively group of proteins are functioning for conclusive response of a particular function. Also, we have observed that the first module is involved mainly in biosynthetic process and is highly densed with comparatively high clustering coefficient among all the modules. These above findings may be useful for the researchers who are working in the area of proteomics and pharmaceutical research.

KEYWORDS : Protein-Protein Interaction, clustering coefficient, power law

INTRODUCTION

Clostridium tetani (C. tetani) is a causative means of tetanus disease which is characterized by rigidity and powerful spasms of skeletal muscles (Stock 2015). The muscles stiffness usually involves the jaw (Lockjaw), neck and then whole body. In most industrialized countries tetanus is a rare disease due to proper immunization, however in tropical and sub tropical countries with low vaccine coverage and poor medical care, it's also a widely distributed (Stock 2015).As per the WHO report which estimated up to 290,000 people worldwide die. Africa is having the maximum death due to occurring the disease. In fact, the WHO reports it is the second leading cause of death from vaccine preventable disease.

*C. tetani, th*e bacteria which is responsible for tetanus was discovered by Nicolauir (1884). It is found in soil, human feces or in the gastrointestinal tract of animal spore () .It can cause infection by contaminating wounds (Bruggemann, Baumer et al. 2003).*C. tetani* produces two neurotoxins (i) Tetanolysin and (ii) Tetanospasmin. Tetanolysin is a hemolysin with no recognized pathogenic ability while tetanospasmin is the peptide which is responsible for tetanus.

The total genome of *C. tetani* consists of 2,799,250 bp encoding with 2,372 ORF. The tetanus toxin is encoded on a 74082 bp plasmid which consists of 610 ORF. The G-C content of *C. tetani is* 28.6%, while the G-C content of plasmid PE88 encoding the toxin TeTx is 24.5% (). The *C. tetani* plasmid PE88 has the gene TeTR for TeTx and it's also have directly transcriptional regulator. TeTx is present just downstream from the TeTR gene() It was thought that the TeTR gene may be (+) regulator of expression of toxin but recent work shows that TeTR actually is a sigma factor of a subgroup that is unique to *clostridium sp*. Due to the presence of RNA polymerase enzyme, TeTR is attached to the core enzyme and enhances the transcription of the target DNA at the promoter (). Some other regulatory genes are also present on the plasmid like TP_{05}, CTP_{10}, CTP_{11}. These regulatory genes are the sigma factors like protein which do not involve in the regulation of toxin production like TeTR (). In addition to the main tetanus toxin TeTx, PE88 plasmid also encodes other virulence factors like collegenase (ColT) is an enzyme which can break down collagen also ColT helps to destroy tissue in infected host which is the most abundant protein in our body.

The main chromosomes of *C.tetani* also have genes responsible for virulence factor. Genes responsible for tetanolysin O, hemolysin, fibronectin-binding protein, S-layer protein have been found (). It is still unclear how the origin of the plasmid (PE88) takes place. More than 50% of the ORF are unique only for *C. tetani ()*. Most of the toxin genes are present on the plasmid are capable of transduction by virus in most of the *Clostridium sp. ()*.

C. tetani enter into the host through wounds found in the skin. Once an infection is established, it produces two neurotoxins (i) tetanolysin and (ii) tetanospasmin.Eleven strain of *C. tetani* have been recognized, which differ mainly in flagellar antigens and in their capacity to produce tetanospasmin. The genes for toxin production are encoded on a plasmid which is present in all toxigenic strains and all the strains are capable of producing identical toxin.

Under anaerobic condition in infected tissue, tetanolysin is capable of damaging the local live tissue and making the condition good for bacterial multiplication (). Tetanospasmin is the main toxin of tetanus. It consists of two chain polypeptide having 150 KD Dalton. It's cleaved by a protease enzyme into two fragments: fragment A (50 KDa"light chain") and fragment B (100 KDa heavy chains") which are connected to each other by disulfide bridges. The carboxyl terminus of the heavy chain binds to the neural membrane and the amino terminal facilitates cell entry().

Tetanus toxin is seize up into nerve terminals of lower motor neurons, the nerve cells that activate voluntary muscles (). That is because the initial symptoms of tetanus is flaccid paralysis, caused by intervention of acetylcholine vesicle related at the neuromuscular junction (). Tetanus toxin moves in the axons of lower motor neuron and finally reaches to the spinal cord or brain stem ().Then the toxin moves across synapses and taken up by the nerve end of inhibitory GABA and glycine neuron that controls the lower motor neurons(). After moving into the nerve ending, tetanus toxin cleaves VAMP inhibits release of GABA and glycine().

The whole map of protein interaction that can arise in a existing individual is called interactome (). Therefore Protein-Protein Interaction (PPI) is the physical contact between highly specified two or more proteins as a result of biochemical event guided by electrostatic forces along with hydrophobic effect. Commonly they are explained as physical contact with molecular docking between protein that occur in a cell or in living organism *in vivo* (). Many PPI in a cell form PPI network, where proteins are nodes and their edges are interaction. It has been discovered that protein with high degrees of connectedness are more likely to be essential for survival than protein with lesser degrees ().

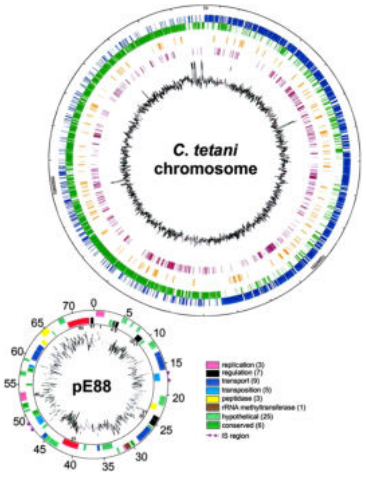

Figure .1: Circular map of the chromosome and the plasmid pE88 of clostridium tetani

There are several reason due to which PPIs is in the light of research.

- PPI greatly helps in the biological research and potentially make the discovery of novel drug target much easier().
- PPI help to understand human disease on a system wide level().
- PPI is an effective tool in solving various biological problem in signal transduction, gene regulation and metabolism().

Approach	Technique	Summary
	Tandem affinity purification-mass spectroscopy (TAP-MS)	TAP-MS depend as soon as possible labeling of the protein of interest on its chromosomal locus, trailed by a two-stage cleaning procedure and mass spectroscopic examination
	Affinity chromatography	Affinity chromatography is highly responsive, can even distinguish weakest connection in protein,futhermore tests all the example proteins similarity for communication
	Coimmunoprecipitation	Coimmunoprecipitation affirms interaction utilizing an entire cell extricate where proteins are available in their native shape in an unpredictable blend of cell parts.
	Protein microarrays (H)	Microarray-based study permits the concurrent examination of a large number of parameters on a single experiment
	Protein-fragment complementation	Protein-fragment complementation assays (PCAs) can be utilized to distinguish PPI between proteins of any atomic weight and express at their endogenous levels.
	Phage display (H)	Phage-display approach originates with the fusion of protein and hereditary apparatus in a solitary phage particle
	X-ray crystallography	In X-ray crystallography we can visualize the structure of protein at atomic level and also it increases the understanding of protein function and interaction
	NMR spectroscopy	In NMR spectroscopy we can detect weak protein-protein connection
In vivo	Yeast 2 hybrid (Y2H) (H)	Yeast two-hybrid technique is commonly settled out by determination a protein of significance against an arbitrary library of probably protein cohorts
	Synthetic lethality	The main focus of synthetic lethality is the functional interaction instead of physical interaction
In□□silico	Ortholog-based sequence approach	In Ortholog-based sequence the PPI is predicted by the homology of inquiry protein in the protein databases using local sequence algorithm approach
	Domain-pairs-based sequence approach	In Domain-pairs-based approach the protein protein interaction is predicted on the basis of domain domain interaction
	Structure-based approaches	The protein protein interactions can also be predicted through the structural similarities of two proteins
	Gene neighborhood	If gene neighborhood of multiple organism is conserved then it may be possible that they have same functionality between the proteins encoded by the associated genes
	Gene fusion	Gene fusion, is also known as Rosetta stone method which depends on the idea that if a large number proteins have a single domain in one organism then these can be fused in another organism to form multidomain protein
	Insilico 2 hybrid (I2H)	The I2H method depends on the fact that if the no. of protein interacts with each other then it must undergo co evolution so to make the protein function authentic
	Phylogenetic tree	In phylogenetic tree technique the evolutionary history of the protein is the main basis for assuming the protein-protein interaction
	Phylogenetic profile	If the two proteins have the similar Phylogenetic profile then there is a chance that they will interact with each other.
	Gene expression	The basis for Gene expression is that if the protein of the gene having the same expression profile cluster then there is forecast that they will interact with each other.

Graph theory can reveal hidden attributes of a network ()**.** Currently, methods in molecular biology and high-throughput technologies has made possible to study a large set of genes at once along with enabling the study of larger genetic networks.

The degree of a node is defined as the number of connections it has with other nodes. *In-degree* is the number of edges pointing to itself (node). *Out degree* It is the number of edges going out of node.

High degree of a node implies that, it has high connectivity. It is the degree distribution which reports the nodes that are highly connected in the network, and can be module.

Degree distribution is a global characteristic, while degree tells about the relationship of a specific node to other node in a network. Degree distribution computes the probability distribution of these degrees for the whole network.

The probability degree distribution P(k), for a network may be defined as the fraction of nodes present in network with degree *k*. As a result, if there are total *n* nodes in the network and n_k nodes of them having degree *k*, then
$P(k) = n_k/n$

Arifuzzaman et al. constructed the PPIN of E.coli having 16050 interactions by 2667 protein, including 798 uncharacterized proteins () and about 521 common proteins is found between both the protein interaction network. The common proteins were connected by 3088 and 5030 interaction in the two networks. However, only 218 common interactions in those inter**action.** However, Arifuzzaman network have many interaction with high essential protein which support the reliability of the network (). Yeast is the best characterized organism nearly 90% of the protein-protein interaction is identified (;). Ito et al. () constructed the PPI of 4549 interaction containing 3278 protein. The largest sub network contain 94% interaction and 87%protein.Ho et al. () reconstructed the 3617 PPIN of 1578 protein by high throughput mass spectroscopic protein complex identification(HMS-PCI)and obtained a sub network of DNA damage response and 2 sub network of signaling pathway. However more and more study of protein-protein interaction will give you a comprehensive analysis of other organism.

Several PPIN of *Plasmodium falciparum* were constructed based on *in vivo* () or *In silico* method (**;;)**. The focusing area in the construction of PPI of animals are *Homo sapiens* (; ;) *Drosophila melanogaster* (**;)**and C.elegans()**.** First protein interaction of D.melanogaster is reconstructed by Giot et al. () which contains 4780 interaction among 4679 protein. Second PIN was constructed by Guruharsha et al.() which is large scale Drosophila Protein Interaction Map (DPiM) having 2297 protein and 10969 high confidence co-complex interaction. As it has been already revealed that disturbance in human protein interaction network leads to many diseases. So the complete information of human protein interaction network is very important to understand the disease. Rob et al. () reconstructed another *Homo sapiens* PIN having 6643 interaction & 2235 protein within deep extract of this dataset. They revealed some new and former concealed protein-protein interaction and connection linking pathways. This study is the crucial pace to learn human disease in the fortune.

*A lot of work has been done to study the function of PPI in the life cycle of virus() especially phage.Coliphage is the best studied temperate phage. More than 10 research group have made the PPI between and its host i.e. E.Coli()***, ()** *Rajagopala et al. () found 97 interaction among 68 protein by using yeast 2 hybrid interactome and from that 16 protein were already known. Blasche,S et al. () further combined the phage with their host E.coli. He got the interaction containing 631 connections and after further screening and retesting 62 high confidence interactions is found. This study exposes the new interaction occurring between the Euclid transcriptional network and protein which is very important to understand the biological regulation. T7 phage was the focus of the 1st genome wide screen for phage PPI using the Yeast 2 hybrid assay. ( ) 55 proteins are found containing 43 interactions out of which 15 between the phage and its virus. Recently the research on pathogen-host interaction attract many interest with several virus-human PIN reconstructed (; ;) De Chassey B et al. 2008 constructed interaction between hepatitis C virus(HCV) and human protein. Nearly 314 PPI is obtained using yeast 2 hybrid and 170 is extracted by literature. Incorporation of this data set with the already constructed human interactome show that the protein interacting with hepatitis C virus are complement in highly central and interconnected protein. Another research group Calderwood M.A et al. 2007 reconstructed the PIN of Epstein-Barr virus and also the interaction of Epstein Barr virus (EBV) protein with the human protein. As a result he found that 43 interaction between EBV protein and 173 interactions between EBV protein and human protein by using high throughput yeast 2 hybrid systems.*

In this work, we first constructed the Protein-Protein Interaction network of ***Clostridium tetani.*** Secondly, detecting the different modules in the interaction network which is further analysed using various graphical approaches.

Methods

We have constructed the network by searching the NCBI database for the genome of clostridium tetani (NCBI ID:1098) it contain 2718 genes transcribing 34000 protein. We then extracted all the genes from the genome. After removing hypothetical and putative genes from the data, we left with about 2416 unique genes from that we download the list in the form of tabular format. Then we have cross checked these genes from the genes which is presented in KEGG databases. We have got 2506 genes in KEGG databases associated with the genome. We then finally compared two lists of genes from both the sources and found common genes to start our curation from the literature. From this list we have tried to extract those genes which are associated with the infection of clostridium tetani. After manual curation from Pubmed, Genecards we get a list of 50 seed genes. After which we stopped the searching of genes (as we require minimum number of genes to make network) we subjected these 50 genes to the Agilent literature search application in Cytoscape 3.3.0.

In parallel with this we also subjected the list of genes to String databases for consortium of network on evidence basis finally from both these formed network we removed interspecies connection using ID and merged both the network to get a final network of 868 nodes and 2535 edges. The flow chart for the synthesis of network of clostridium tetani is given in fig 3.1.

Software/Tools Used

UniProt provides tools to help with protein sequence analysis and their annotations. Also gives connection with more than 150 other biological databases to help you to get extra data in more specialized condition.

STRING(Search for The Retrieval of Interacting Genes/Proteins) is a biological database, which is a consortium of EMBL, SIB, UZH. It plans to improve access the data by giving a complete, quality controlled accumulation of Protein -Protein interaction for a large number of organisms.

KEGG is a collection of databases dealing with genomics, biological pathway, disease, drug and chemical substance.. KEGG is an incorporated database that consists of 16 databases which are divided into 4 categories: Systems, Genomics, Chemicals and Health Information.

Agilent Literature Search Software is a meta-search tool to mines scientific Literature for finding publication related to search terms and to create biological interaction network based on search result (genes/Protein).

Cytoscape is openly accessible Software Project for coordinating imagining and investigating biomolecular interaction network().

M-Code (molecular complex detection) is a Cytoscape plugin which can find Modules (Highly interconnected region) in a network. It can detect thickly associated areas in substantial protein- protein connection arrange which can represent molecular connection().

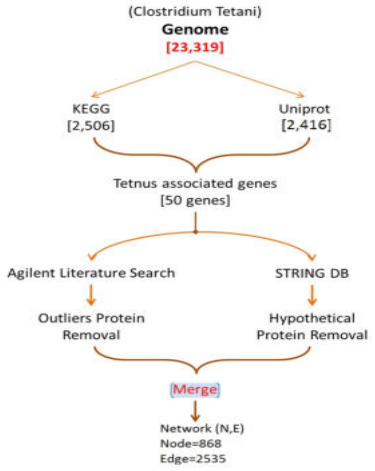

Figure 3 . : Flow chart for Synthesis of PPI of Clostridium tetani

RESULT AND DISCUSSION

We have constructed the network by taking node as protein and edges as interaction between protein. The network has been constructed with the method given in material and method section, We have found 868 nodes and 2535 edges (Fig 4.1)and single connected component. This network is undirected because there is no meaning one indirect with other and other does not interact with one.

The network is sparse due to the density of the network is 0.007 which is too much small as compared to the other network. This sparseness of the network depicted either all the PPI are not fully studied or physical interaction behavior is not in fact frequent. The network does not have lot of cluster due to it have low (0.242) average clustering coefficients.

The constructed protein-protein interaction network depicts that there are very less number of protein that there interacting proteins are interacts with each others. Average path length is also much high (4.441) and therefore, all proteins are not interact directly to each other, although even this implies that there are not too much proteins whose are simultaneous interaction with two protein. There are 17 proteins found whose are interact to itself to do some biological process.

Table 4.1: Global Characteristics of PPI Network

Average Clustering Coefficient	0.242
Network Diameter	10
Network Radius	5
Network Centralization	0.031
Shortest Path	752556
Path Length	4.441
Average no. of neighbor	5.841
Number of nodes	868
Network density	0.007
Network heterogeneity	1.149
Self loop	13

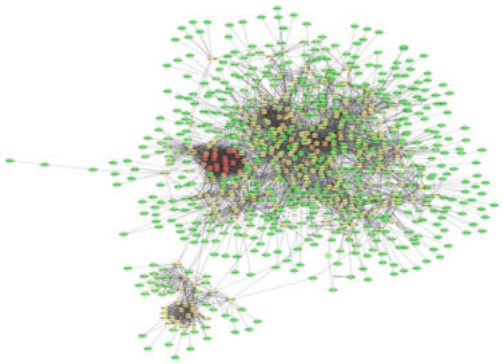

Figure 4. : PPI Network of Clostridium tetani

Table 4.2: Top ten Genes with high Degrees

S No.	Name of Protein	Protein with high degree	Average Clustering coefficient
1	Q897L2	157	3.52018454
2	Q897K3	153	0.70355731
3	Q897J8	150	0.74025974
4	Q897K1	149	0.70995671
5	Q897J6	148	0.74285714
6	Q897K2	147	0.67588933
7	Q897J7	146	0.78095238
8	Q897J9	145	0.75714286
9	Q897J5	145	0.76190476
10	Q897L4	139	0.57142857

These proteins have important role in the biological process of the bacteria. The number of interaction of the top ten proteins is given in table 4.2. The detail description and function of the top ten proteins is given below.

CobD The product of this gene is aminotransferase CobD. This enzyme is involved in the transfer of an alpha amino acid to an alpha keto acid. This amino group usually bond to the prosthetic group Pyridoxal phosphate(vitamin B6).

CbiA The outcome of this gene is cobrinate a,c-diamide synthase.It catalysis the conversion of cobyrinic acid to cobyrinic acid a,c-diamide via the intermediate formation of cobyrinic acid c-monoamide**.**

CTC 00736 The result of this gene is Precorrin-3 methylase which is involved in the synthesis of cobalamin (vitamin B12)Which is water soluble vitamin characterized by the Possession of Corrin nucleus containing a Cobalt atom.

CbiD The outcome of this Gene is cobalt-precorrin-SBC(1)-methyltransferase.This Protein is involved in the synthesis of cob()yrinate a,c-diamide from sirphydrochlorin and also involved in the formation of corrin. Corrin is an important part of vitamin B12 molecule.

CTC 00732 The Product of this gene is Precorrin 8X methyl mutase protein**.**

CbiG The outcome of this gene is cobalamin biosynthesis protein**.**

CTC 00735 The result of this is Pre-2 C 20 methyltransferase protein. This protein is involved in the synthesis of corrin containing a cobalt atom**.**

CTC 00739 encodes a protein called precorrin-3BC17 methyltransferase.This protein is involved in the synthesis of cobalamin(vitamin B12).

CTC 00740 The outcome of this gene is Precorrin-6x reductase protein and is involved in the biosynthesis of cobalamin.

CobQ Cobyric acid synthase and is involved in both glutamine metabolic process and in biosynthesis of cobalamin.

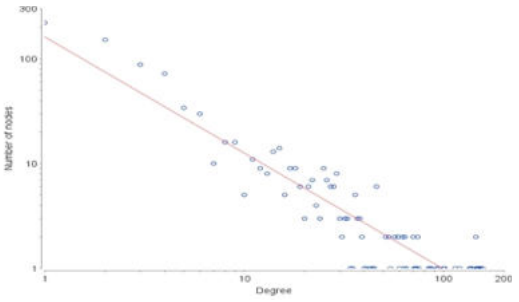

Figure 4. : Node Degree Distribution

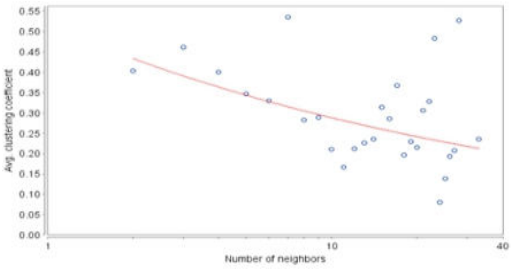

Figure 4.3: Average clustering coefficient

A general property of numerous bulky networks is degree distribution. It has been found that are few protein which are highly connected, and rest of the protein are less connected with the most of the real network follows the scale free power law distribution (). This PPI follow scale freeness property with exponent value γ=-1.107 Therefore, there others. This character also reflected the robust nature of the network. Thus, these proteins may be targeted for the researcher of pharmaceuticals. Clustering coefficient means clustering of network. If the clustering coefficient of network is 1 it indicate high clustered neighbor of a node while 0 represent no cluster of a node. Average clustering coefficient gives the global characteristic of clustering.

Another, important characteristics of organization of protein in the organism is hierarchal structure of the network().As we have discuss the clustering coefficient of whole network as a single value. Now, question how clustering coefficient distributed throughout the network? Figure 4.3 showing degree vs. average clustering coefficient of all nodes whose degree is fix. Again, it has seen that in homogeneously of clustering have been found with taking different degree nodes. In other way, we can say that few low degree nodes have found high clustering and other high degree node found low clustering. This character depicts about the interaction among the group of protein.

Identified modules and their biological significance

To investigate the modular composition within the constructed PPI network of clostridium tetani, we employed M-Code, a Cytoscape plugin and concluded 10 total number of modules. We further choose top 5 modules for our study.

The first module has 18 genes with 140 number of interactions (can be seen in fig 4.5).The predicted function of proteins of module 1 distribution is based on GO(Gene Ontology). The output depicts that the highest degree protein in the module has 28 interaction,.526 clustering in and is involved in cobalamin biosynthetic process and has Protein methyl transferase activity. As most of the protein in the module which have high degree is involved in cobalamin biosynthetic process and are performing methyl transferase activity.

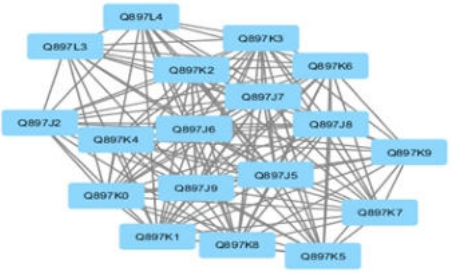

Figure 4. : Module 1 with 18 node and 140 edges

The second module has 16 genes with 100 number of interaction (as can be seen in fig4.6). The predicted function of proteins of module2 distribution is based on GO. The output depicts that the highest degree protein in the module has 33 interaction,.23 clustering coefficient and is involved in the 'de novo 'Pyrimidine nucleobase biosynthetic process with the amino acid binding molecular functior

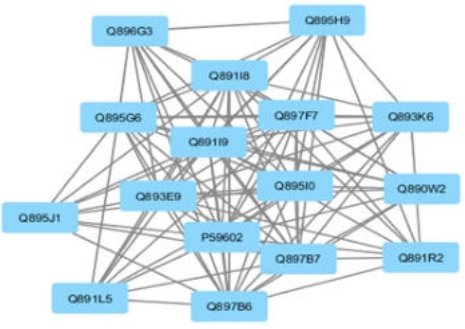

Figure 4. : Module 2 with 16 node and 100 edges

The third module has 9 genes with 33 number of interactions (can be seen in fig 4.7).The predicted function of proteins of module 1 distribution is based on GO. The output depicts that the highest degree protein in the module has 28 interaction,.526 clustering in and is involved in cell division process and has ATPase activity. As most of the protein in the module which have high degree is involved in cell division and protein folding process and are performing ATPase activity and protein folding.

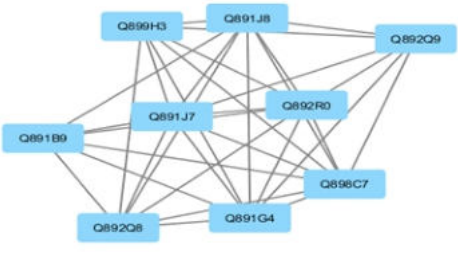

Figure 4. : Module 3 with 9 node and 33 edges

The fourth module has 17 genes with 17 number of interactions (can be seen in fig 4.8).The predicted function of proteins of module 1 distribution is based on GO. The output depicts that the highest degree protein in the module has 28 interaction,.526 clustering in

and is involved in Aromatic amino acid biosynthetic process and has ATP binding activity. As most of the protein in the module which have high degree is involved in Aromatic amino acid biosynthetic process and are performing ATP binding activity.

Figure 4. :Module 4 with 17 node and 17 edges

The fifth module has 8 genes with 23 number of interactions (can be seen in fig 4.9).The predicted function of proteins of module 1 distribution is based on GO. The output depicts that the highest degree protein in the module has 28 interaction,.526 clustering in and is involved in Transporter activity and has ATPase activity. As most of the protein in the module which have high degree is involved in Transporter activity and are performing ATPase activity.

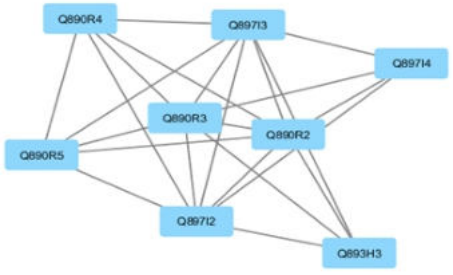

Figure 4. 8:Module 5 with 8 nodes and 23 edges

CONCLUSION

In this study we used a graph approach to analyze the protein - protein interaction network of Clostridium tetani. The constructed PPI also follows scale free characteristic as well as hierarchical organization as followed by the other biological networks. Thus the interactions among the proteins in the network follow heterogeneity with the other proteins in the bacteria due to the specific nature and characteristics of some of the proteins, whereas the random interactions among proteins follow homogenity.The interactions of proteins also follow the modular hierarchical nature which represent low interactive proteins are much modular than higher interactive protein. This happens due to cohesively group of proteins are functioning for conclusive response of a particular function.

The second interesting point is the identification of modules in the network based on the assumption that individual proteins do not involve in the major activities. But a group of proteins are in coordinated fashion to bring about some specific functions or activity of the organism. Therefore, we have detected modules throughout the constructed PPI. We have observed that the first module is involved mainly in biosynthetic process and is highly dense with comparatively high clustering coefficient among all the modules.

These findings may be useful for the researchers who are working in the area of proteomics and pharmaceutical research.

Acknowledgment

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