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MODULE BASED PROTEIN INTERACTION NETWORK ANALYSIS OF BACTERIAL UNIVERSAL STRESS PROTEIN

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ABSTRACT: The Universal stress protein appears to belong to all stress and starvation stimulons under the inclusive regulation of gene expression. It has been suggested that Usp proteins have different physiological functions that reprogram the cell towards defense and escape during cellular stress. In this study Protein interaction network (PIN) analysis was used to predict its mechanism of molecular action. The PIN of Universal stress protein was constructed by Cytoscape and the function modules identified by gene ontology (GO) enrichment analysis based on molecular complex detection (MCODE). The PIN of Universal stress protein has scale-free, small world and modular properties. Based on analysis of these function modules, the mechanism of Universal stress protein is proposed. The protein-protein interactions information of Universal stress protein is connected to the protein secretion pathway, apoptosis, cell division, cell shape and gene regulation. These results will serve as a distinctive resource to present a physical map of protein profile of bacteria under stress condition, drug targeting and the development of novel therapeutics.

KEYWORDS: Bacterial Universal stress protein, Protein interaction network, Module, Gene ontology enrichment analysis, Molecular complex detection, Cytoscape.

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1. INTRODUCTION

The universal stress protein superfamily encompasses a conserved group of proteins that are found in bacteria, archaea and eukaryotes. Universal stress protein (USP) acts as a precursor in constituting a natural biological defense mechanism under stress conditions such as the presence of oxidants, uncouplers, DNA damaging agents, nutrient starvation, heat shock or other stress agents that could cause arrest of cell growth. The levels of universal stress proteins become elevated in response to a variety of stress conditions including antibiotics. It has been reported that universal stress proteins have functional roles in adhesion, motility and oxidative stress resistance. However, some forms are not directly involved in stress resistance but are essential. It has been suggested that Usp proteins have evolved different physiological functions that reprogram the cell towards defense and escape during cellular stress. Notably, the functions provided by Usp proteins, oxidative stress defense, iron homeostasis, and motility/adhesion are known to be essential in bacterial pathogenesis. Based on structural analysis and their amino acid sequence, the Usp proteins have been divided into different classes. Most organisms have multiple paralogs of USPs, where the number of copies depends on the organism. Usp genes including uspA, uspC, uspD and uspE respond to stress conditions causing growth arrest and under extreme conditions production of Usp proteins are repressed. The bacterial usp genes usually encode either a small Usp protein (around 14-15 kD) that has one Usp domain, or a larger version (around 30 kD) consists of two Usp domains in tandem. [1-4]. In responses to different disturbances and circumstances, the physical binding of two or more proteins is referred as Protein–protein interaction (PPI) that provides considerable adaptability for biological cells to adapt to the changing environmental conditions. [5] Based on the PPIs, more systematic protein networks were established gradually, known as the protein-protein interaction network (PIN). Similar to most biological networks, PIN are scale-free and small-world properties. [6] Scale-free represents that the connectivity distribution of nodes in a network fits a power law. The scale-free property indicates that a PIN consists of a few highly-connected proteins (hub proteins) and a large amount of less-connected proteins, which makes a network endure a random protein removal, but susceptible to the removal of hubs. [7] Small-world indicates that any two nodes in a network can be connected with a small number of links, while the average path length between nodes in PINs is much shorter than a random network due to the existence of hub proteins. PIN is a major component of interactomes, which also include other molecular interactions in the cell, such as genetic interactions. [8] Most commonly, interactome refers to the PIN or its subsets. Furthermore, PIN is an effective tool for understanding the complex world of biological processes inside the cell and solving various biological problems in signal transduction, gene regulation, and metabolism. [9] Given the significant importance of PINs, proteome-wide interaction networks have been studied in many organisms from prokaryote [10] to eukaryote [11], from unicellular [12] to human [13]. The technique of collecting protein interaction datasets for reconstructing a PIN is improving and the applications of PINs have spread into more

and more areas of the biological research. Moreover, the analysis based on PINs leads to accumulation of massive amounts of data concerning protein interaction pairs, protein complexes, and protein functions. The biological hypotheses deduced from PINs play a major role in guiding scientists to understand further the mechanism in cells and design more reasonable experiments for investigating the mystery of protein systems in various organisms. However, it has become possible to combine the traditional study of proteins as independent entities with the analysis of large protein interaction networks. [14, 15]. This is of particular interest as many of the properties of complex systems seem to be more closely determined by their interactions than by the characteristics of their individual components. The study of protein interactions is important not only from a theoretical perspective but also in terms of potential practical implications because it might enable new drugs to be developed that can specifically disrupt or modulate protein interactions, instead of simply targeting a given protein's complete set of functions. [16,17]. As the role of functional dysregulation of PPIs as the underlying cause of disease is well understood, network pharmacology that advocates combination therapies targeting multiple interconnected nodes in a PPI network represents a new setting for disease treatment.

2. MATERIALS AND METHODS

Network Construction:

To construct the Protein Interaction Network for bacterial Universal Stress Protein, we retrieved interaction networks from BioGrid, IntAct and Uniprot databases and merged them. These databases are protein-protein, protein-small molecule, protein-nucleic acid interactions that integrate many sources of experimental and manually-curated evidence with text mining information and interaction prediction. The PPI information was also obtained from STRING [18] and Interactome3D [19] servers that are used to retrieve the predicted interactions. All associations available in STRING are provided with a probabilistic confidence score. Targets with a confidence score greater than 0.7 were selected to construct the PPI network. [20, 21]

Network analysis:

Topological properties are important tool to gain an insight into the structure and the organization of the resultant large complex networks. Therefore, topological parameters such as the connected components, clustering coefficient, degree distribution, and average shortest path were analyzed by Network Analyzer [22] in Cytoscape [24] software. In contrast to the random network, the properties of scale free, small world and modularity of the PIN were also investigated based on the topological parameters. The Cytoscape generates a grid view of protein-protein interactions which are arranged according to their individual blast scores. The edges in all PPI networks were treated as undirected.

Fitting a line:

Network Analyzer provides another feature - fitting a line on the data points of some complex parameters. The method applied is the least squares method for linear regression. [25] Network

Analyzer gives the correlation between the given data points and the corresponding points on the fitted line. In addition, the R-squared value (also known as coefficient of determination) is reported. Fitting a line can be used to identify linear dependencies between the values of the x and y coordinates in a complex parameter shows the fitted line on a neighborhood connectivity distribution. The correlation between the data points and corresponding points on the line is approximately 0.969. The R-squared value is 0.939 giving a relatively high confidence that the underlying model is indeed linear.

Fitting a power law:

The degree distribution of many biological networks approximates a power law: DD (k) ~ kα for some negative constant α. Network Analyzer can fit a power law to some topological parameters. Network Analyzer uses the least squares method [25] and only points with positive coordinate values are considered for the fit. This approach fits a line on logarithmized data and gives the correlation between the given data points and the corresponding points on the fitted curve. In addition, the R-squared value (also known as coefficient of determination) is reported. This coefficient gives the proportion of variability in a data set, which is explained by a fitted linear model [26, 27]. Therefore, the R-squared value is computed on logarithmized data, where the power-law curve: $y = \beta x^a$ is transformed into linear model: 1n y = lnβ + alnx. The MCODE was used to further divide the PPI into clusters or modules, using a cutoff value for the connectivity degree of nodes (proteins in the network) greater than 3. [23] The algorithm has the advantage over other graph clustering methods of having a directed mode that allows fine-tuning of clusters of interest without considering the rest of the network and allows examination of cluster interconnectivity, which is relevant for protein networks. [23] Based on the identified modules, GO functional annotation and enrichment analysis were performed using the BinGO [24] plugin in Cytoscape with a threshold of $P < 0.05$ based on a hypergeometric test.

3. RESULTS AND DISCUSSION

Network Construction:

The PPIs of bacterial Universal stress protein was imported in Cytoscape, union calculations were carried out and the duplicated edges of PPIs were removed using Advanced Network Merge plugins, and the largest connected subgraph was selected as the PIN of bacterial Universal stress protein, which included 5872 nodes and 8224 edges as shown in Fig. 1. The nodes represent proteins and edges indicate their relations. The gray nodes represent seed nodes and the others are nodes that interact with seed nodes. Due to limits of the current studies, some protein interactions are still uncertain. As a result, the network constructed for this study is not inclusive and the largest connected subgraph was selected for further analysis.

Network Analysis:

Topological analysis:

All the topological parameters were calculated, as shown in Table 1.

Degree distribution:

Degree distribution was computed by counting the number of connections between various proteins of the network. [28,29] As shown in Fig. 2, the degree distribution of the PIN of bacterial Universal stress protein followed the power law distribution and the equation is (for In and Out degree distribution: $y=ax^b$; where, a= 2577.1, b= -2.361, correlation = 0.993, R-squared = 0.897 and a= 880.25, b= -1.761, correlation = 1.000, R-squared = 0.871 respectively). The PIN of bacterial Universal stress protein is a scale-free network.

Table.1. The topological parameters of the protein interaction network of bacterial universal stress protein

Parameter	Value	Parameter	Value
Clustering Coefficient	0.075	No. of nodes	5872
Connected components	503	No. of edges	8224
Network diameter	30	Network density	0.0
Network radius	1	Network heterogeneity	1.796
Network centralization	0.021	Isolated nodes	36
Shortest paths	720852 (2%)	No. of self loops	276
Characteristic path length	8.153	Multi edge node pairs	0
Average no. of neighbors	2.707		

The network diameter is the greatest distance between any pair of vertices. Network centralization is a network index that measures the degree of dispersion of all node centrality scores in a network. Network heterogeneity measures the degree of uneven distribution of the network.

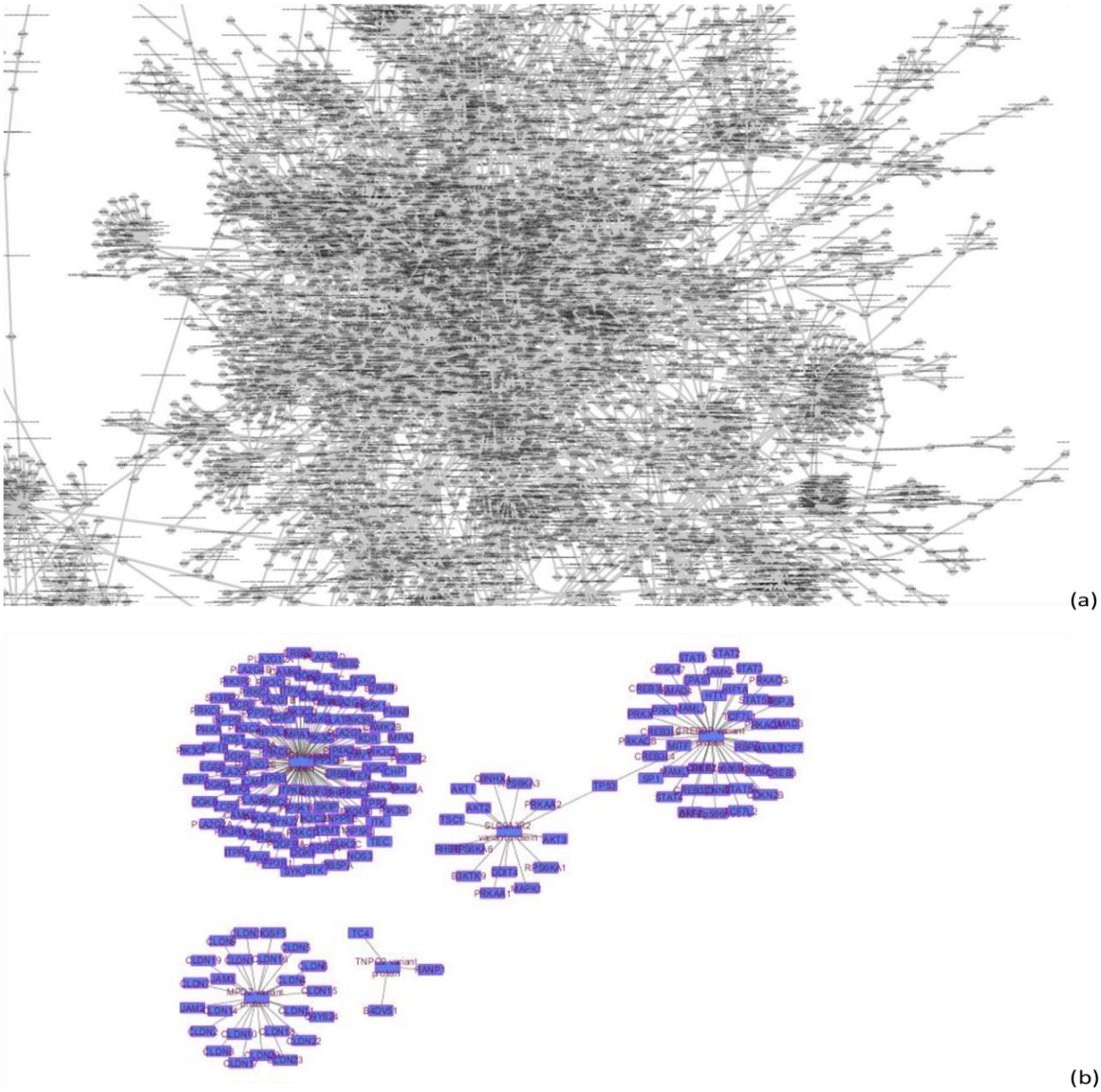


Figure 1. The protein network of Bacterial Universal stress protein. The nodes and edges indicate the proteins and their relationships. The grey nodes represent seed nodes and the white ones are nodes that interact with the seed nodes. Average shortest path refers to the average density of the shortest paths between all pairs of nodes shortest path length between any two proteins is 8.153. This meant that most proteins were very closely linked and the PIN of bacterial Universal stress protein is a small world network. [28, 29]

Clustering coefficient:

Clustering coefficient refers to the average density of the node neighborhoods. [28,29] The value of clustering coefficient of bacterial Universal stress protein interaction network is 0.075. The equation for Average clustering coefficient distribution is $y=ax^b$; where, a= 2.421, b= -1.526, correlation = 0.733, R-squared = 0.671. The higher the clustering coefficient, the more modular the network would be. Compared with a random network whose number of nodes and edges are the same as the PIN of

bacterial Universal stress protein, the PIN clustering coefficient for bacterial Universal stress protein was higher. This indicates that the PIN of bacterial Universal stress protein possesses the property of modularity. This result suggests that the network possesses the scale-free property, a small world property and modular properties.

Neighborhood Connectivity:

The neighborhood connectivity distribution of PPIs; only In, only Out and both In and Out were calculated, when the fitted power law was applied ($y=ax^b$ with values a= 6.546, b= -0.572, correlation = 0.426, R-squared = 0.354 (only In) ; a= 6.171, b= -0.543, correlation = 0.747, R-squared = 0.383 (only Out) ; a= 21.978, b= -0.460, correlation = 0.830, R-squared = 0.551 (both In and Out). The higher correlation was observed for bacterial Universal stress protein.

Stress centrality:

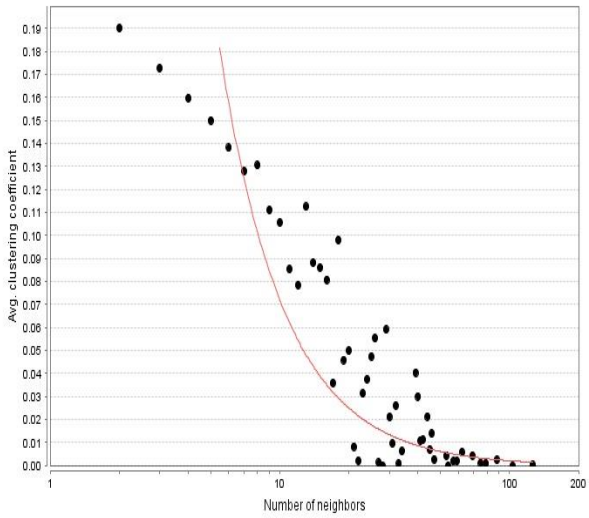
In the stress centrality distribution of bacterial Universal stress protein, correlation and R-squared value was observed when the fitted power law was applied ($y=ax^b$ with values a= 443.23, b= -0.156, correlation = 0.810, R-squared = 0.622). The high correlation value shows a perfect fit of network.

Betweenness Centrality:

In the Betweenness Centrality distribution of bacterial Universal stress protein, the correlation and R-squared value was observed when the fitted power law was applied ($y=ax^b$ with values, a= 0.000, b= 0.216, correlation = -0.225, R-squared = 0.001). The value of correlation and R-squared is very less when compared with the standard (Power law perfect fit correlation = 1.000 and R-squared = 1.000). The betweenness in proteins indicates a non-hub centric organization (co-ordination of modules by non-hub proteins) of the protein interaction networks and in a way supported the idea that some interactions are more important in the network structure. These nodes can represent important proteins in signaling pathways and can form targets for drug discovery.

Closeness Centrality:

Closeness centrality is a measure of how rapidly information spreads from a given node to other reachable nodes in the network. The closeness centrality distribution of the bacterial Universal stress protein is high (Correlation = 0.230, R-Squared = 0.071, by fitted power law $y=ax^b$ with values, a= 0.552, b= -0.255), this shows that highly connected proteins in the network have a pronounced ability to spread information in the network. Nodes with high closeness centrality have potential significance for responding to external perturbations and for maintaining network stabilization. This may be significant because highly connected “hub” proteins usually play essential roles in cell processes [30].



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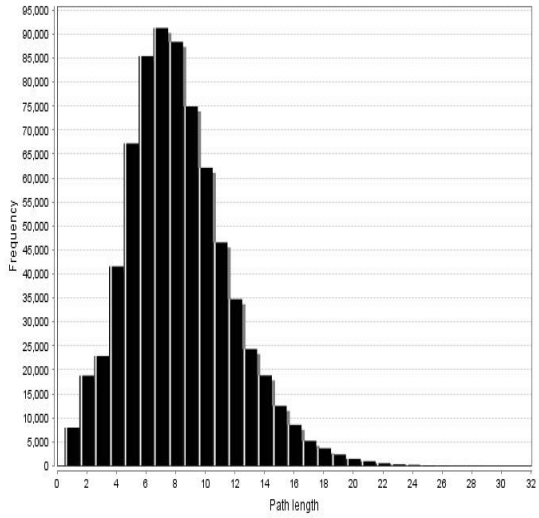

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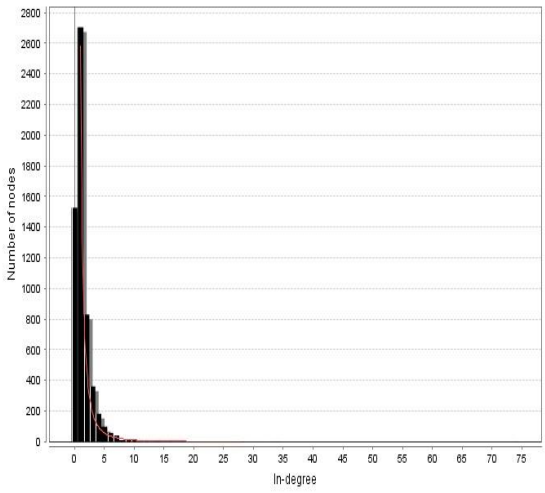

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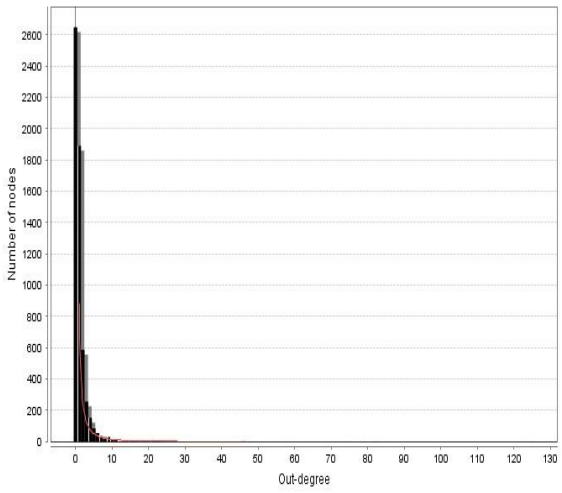

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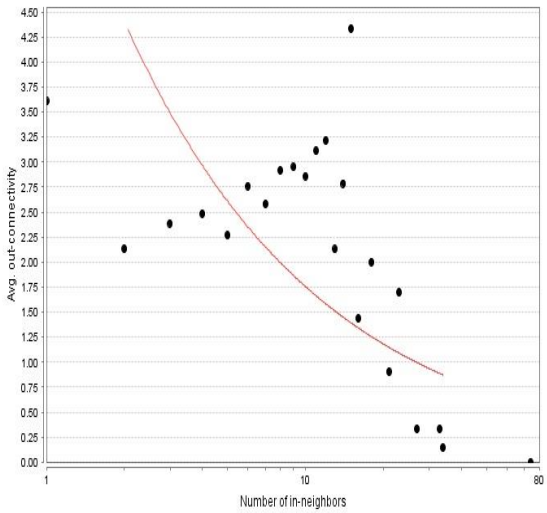

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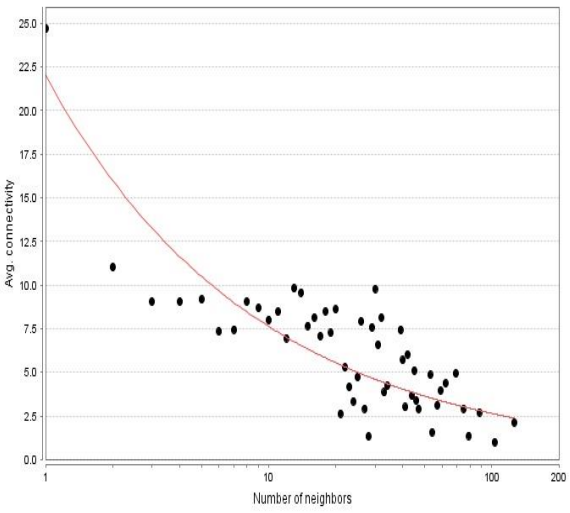

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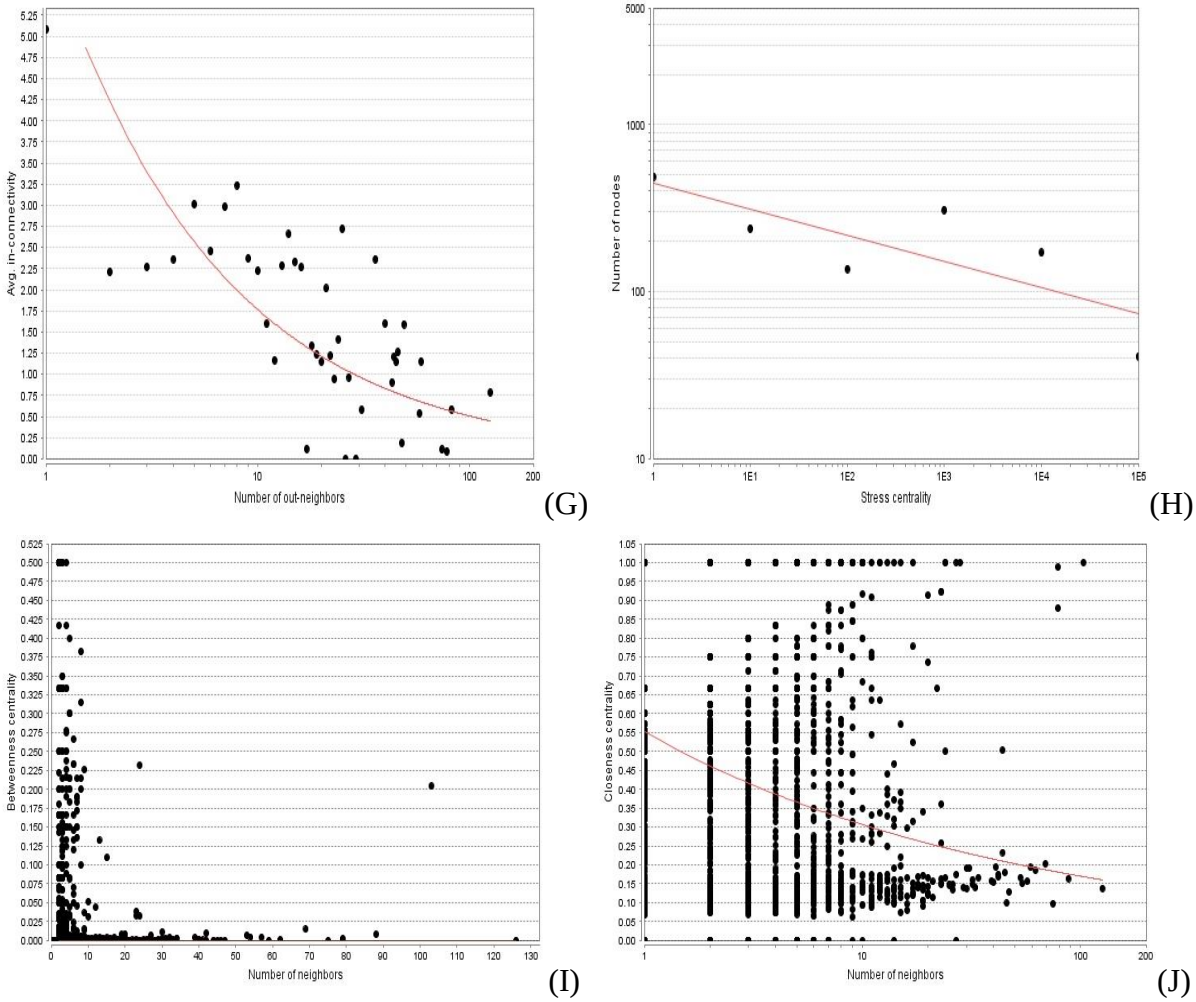


Figure 2. Topological properties of bacterial Universal stress protein PPI network (A) Average clustering coefficient distribution (B) Path length distribution (C) In degree distribution (D) Out degree distribution (E) Neighborhood connectivity (In) (F) Neighborhood connectivity (In & Out) (G) Neighborhood connectivity (Out) (H) Stress centrality (I) Betweenness centrality (J) Closeness central

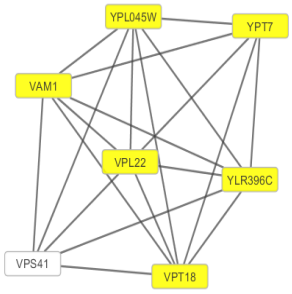
Clustering and GO enrichment analysis:

As shown in Figure 3. 13 modules were identified from the network through the MCODE algorithm. [23] The yellow nodes indicate seed nodes and the other are nodes that interact with seed nodes. The results of functional enrichment analysis using BinGO [24] are shown in Table 2.

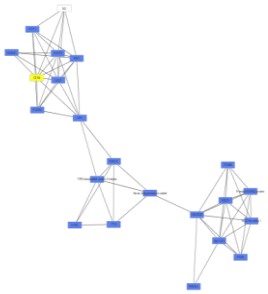
Table. 2. GO biological process terms of the modules

Module	GO term	P-value
Module 1	Regulation of vacuole organization	2.0941×10^{-3}
Module 2	RNA elongation from RNA polymerase I promoter	8.0541×10^{-4}
Module 3	Regulation of nitrogen utilization	1.4497×10^{-3}
Module 4	Retrograde transport, endosome to Golgi	3.3827×10^{-3}
Module 5	Protein retention in Golgi apparatus	1.7719×10^{-3}
Module 6	Nucleolus organization	6.4433×10^{-4}
Module 7	Inositol lipid-mediated signaling	9.6649×10^{-4}
Module 8	Protein secretion	1.2887×10^{-3}
Module 9	Retrograde transport, vesicle recycling within Golgi.	6.4433×10^{-4}
Module 10	Regulation of SNARE complex assembly	1.2887×10^{-3}
Module 11	cis assembly of pre-catalytic spliceosome	3.2216×10^{-4}
Module 12	Mitotic cell cycle spindle orientation checkpoint	9.6649×10^{-4}
Module 13	Tyrosine transport	1.6111×10^{-4}

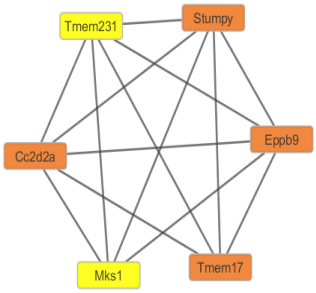
P value is the probability of obtaining the observed effect, a very small P value indicates that the observed effect is very unlikely to have arisen purely by chance, and therefore provides evidence against the null hypothesis.



Module 1



Module 2



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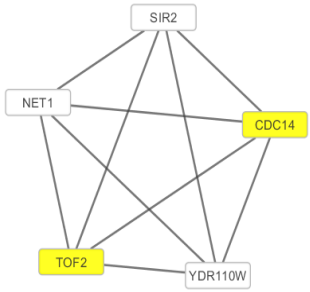
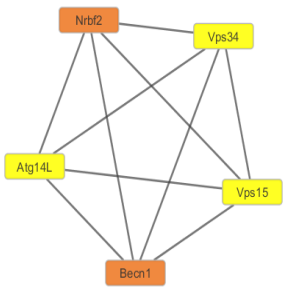


Module 4



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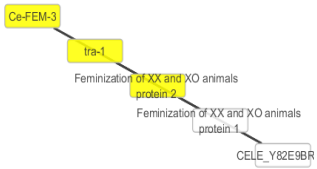

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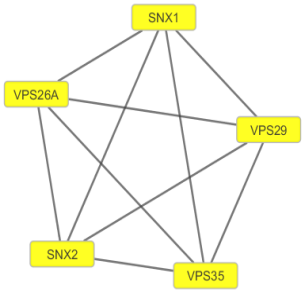
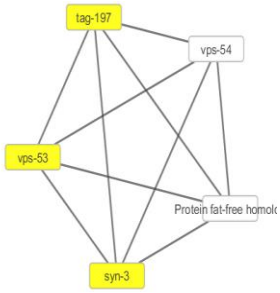
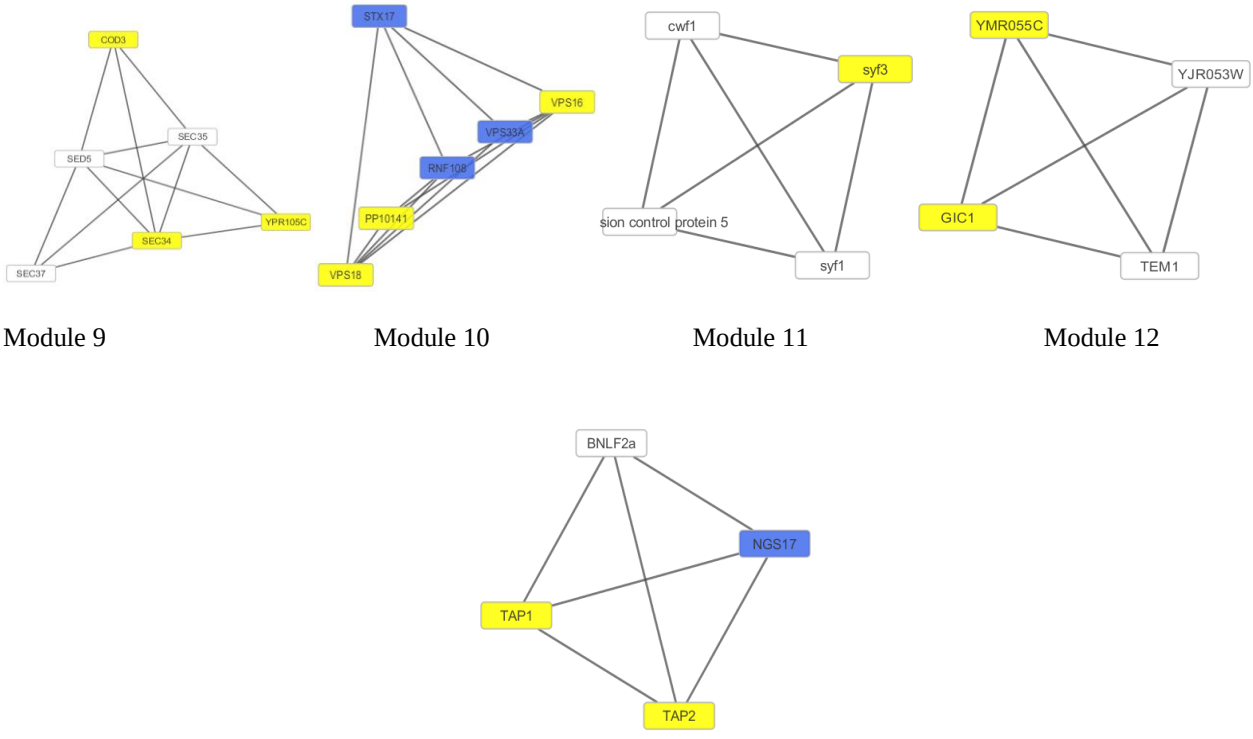


Module 7



Module 8



Module 9 Module 10 Module 11 Module 12

Module 13

Figure 3. Modules in the PIN of bacterial Universal stress protein. With the MCODE algorithm, 13 modules were extracted from the network. The yellow nodes represent seed nodes and their nearest neighbors and the white ones are nodes that interact with the seed nodes.

The results shows that bacterial Universal stress protein has interactions with several biological processes including RNA elongation, Mitotic cell cycle spindle orientation, amino acid transport, Nucleolus organization, pre catalytic spliceosome assembly, protein secretion, regulation of nitrogen utilization, Inositol lipid-mediated signaling, regulation of vacuole organization and protein transport through Golgi complex. Modules 1, 4, 5, and 8, 9, 10 are related to vesicular transport, protein secretion and protein retention in Golgi complex. Module 6 is related to Nucleolus organization and Module 7 is related to Inositol lipid mediated signaling. Module 3 is related to regulation of nitrogen utilization and Module 13 is related to amino acid transport. Module 2 is related to RNA elongation, Module 11 is related to assembly of pre catalytic spliceosome and Module 12 is related to Mitotic cell cycle spindle orientation checkpoint. Module 1 contains proteins such as vacuolar morphogenesis protein (VAM1), vacuolar protein targeting protein (VPT18), vacuolar protein sorting associated protein (VPS41) and (VPL22), and YPT7 that is required for homotypic vacuole fusion. Module 2 contains proteins such as CTR9 that is a RNA polymerase associated protein and SH2 domain binding protein. It has a significant functional role as a component of the PAF1 complex (PAF1C) which has multiple functions during transcription by RNA polymerase II and is implicated in regulation of development and maintenance of embryonic stem cell pluripotency. [31] Module 3 contains proteins

such as Tmem231, a transmembrane protein, which is a component of the B9 complex involved in the formation of the diffusion barrier between the cilia and plasma membrane. Tmem231 is a critical component of a protein complex in the basal body, a ring-like structure that functions in the transition zone at the base of cilia. [32] Mks1 belongs to a small family of B9 domain-containing proteins that also includes B9D1 and B9D2 and all 3 B9 domain-containing proteins associate with basal bodies and primary cilia in mammalian cells.[33] Module 4 contains CDC14; completion of the cell cycle requires the temporal and spatial coordination of chromosome segregation with mitotic spindle disassembly and cytokinesis. [34] It is a protein phosphatase which antagonizes mitotic cyclin-dependent kinase CDC28; the inactivation of which is essential for exit from mitosis. TOF2 (Topoisomerase 1-associated factor 2) which is required for rDNA silencing and mitotic rDNA condensation; stimulates Cdc14p phosphatase activity and biphasic release to promote rDNA repeat segregation; required for condensin recruitment to the replication fork barrier site. TOF2 has a paralog, NET1 that arose from the whole genome duplication. This gene is part of the family of Rho guanine nucleotide exchange factors. Members of this family activate Rho proteins by catalyzing the exchange of GDP for GTP. [35] The protein encoded by this gene interacts with RhoA within the cell nucleus and may play a role in repairing DNA damage after ionizing radiation. SIR2 (NAD-dependent histone deacetylase**)** is a NAD-dependent deacetylase, which participates in a wide range of cellular events including chromosome silencing, chromosome segregation, DNA recombination and the determination of life span.[36] Module 5 contains proteins such as Atg14L (Autophagy related protein 14 like) and VPS 34, VPS15 Phosphoinositide kinase class 3 Serine/threonine-protein kinase required for cytoplasm to vacuole transport and autophagy as a part of the autophagy-specific VPS34 PI3-kinase complex-I. [37, 46] This complex is essential to recruit the ATG8-phosphatidylinositol conjugate and the ATG12-ATG5 conjugate to the pre-autophagosomal structure. It is also involved in endosome-to-Golgi retrograde transport as part of the VPS34 PI3-kinase complex II. [38, 46] Moreover, Module 6 contains fem2, a sex determining protein. It has been suggested that the gene fem-2 plays an important role in regulating a pathway transducing a non-cell-autonomous signal to a nuclear transcription factor. It shows phosphorylation and/or dephosphorylation as a control mechanism in sex determination. Module 6 also contains Ce-FEM3, Feminization of XX and XO animal protein, Tra1 and Her2, Hermaphrodization of XO animal protein 2, essential components of histone acetyltransferase (HAT) complexes, which serves as a target for activators during recruitment of HAT complexes.[39] These proteins are essential for vegetative growth. Module 7 contains maternal embryonic protein VPS35, vesicle protein sorting VPS26A and VPS29 that acts as component of the retromer cargo-selective complex (CSC).[40] The CSC is believed to be the core functional component of retromer or respective retromer complex variants acting to prevent missorting of selected transmembrane cargo proteins into the lysosomal degradation pathway. Module 6 also contains SNX1 (sorting nexin 1); members of this family contain a phox

(PX) domain, which is a phosphoinositide binding domain, and are involved in intracellular trafficking. [41] This endosomal protein regulates the cell-surface expression of epidermal growth factor receptor. This protein also has a role in sorting protease-activated receptor-1 from early endosomes to lysosomes. SNX2 is involved in several stages of intracellular trafficking. It interacts with membranes containing phosphatidylinositol 3-phosphate (PI3P) or phosphatidylinositol 3,5-bisphosphate (PI3,5P2). Module 8 contains VPS53, 54 that act as component of the GARP complex that is involved in retrograde transport from early and late endosomes to the trans-Golgi network (TGN). [42] It also contains TAG197 and SYN 3, synapsins that encode neuronal phosphoproteins which associate with the cytoplasmic surface of synaptic vesicles. [43] Module 9 contains Sec37 and COD3, component of oligomeric golgicomplex 6, complexed with DOR1 and Sec35, component of oligomeric golgicomplex 2. Module 10 contains RING finger protein (RNF108), VPS18 that plays a role in vesicle-mediated protein trafficking to lysosomal compartments including the endocytic membrane transport and autophagic pathways. STX17 (SNAREs), soluble N-ethylmaleimide-sensitive factor-attachment protein receptors, are essential proteins for fusion of cellular membranes. [44,45] Module 11 contains Syf3 complexed with cdc5 protein 4, Cdc5 cell division control protein 5 and Cwf1, Pre- mRNA processing protein 5 complexed with cdc5 (Pre-mRNA-splicing factor prp5) that is required for both cell cycle progression at G2/M and pre-mRNA splicing. It interacts genetically with the PRP4 kinase. [47] Moreover, Module 12 contains protein such as YMR055C, cell cycle arrest protein BUB2, cell cycle arrest protein BFA1 that is part of a checkpoint which monitors spindle integrity and prevents premature exit from mitosis. This cell-cycle arrest depends upon inhibition of the G-protein TEM1 by the BFA1/BUB2 complex. [48] TEM1, GTP-binding protein involved in termination of M phase. TEM1 may play a role in triggering the degradation of G2 cyclin to inactivate M-phase promoting factor at the termination of mitosis. [48] It acts upstream of CDC15 kinase and may be required to activate the CDC15 protein kinase pathway. GIC1, that is required for cell size and shape control, bud site selection, bud emergence, actin cytoskeletal organization, mitotic spindle orientation/positioning, and mating projection formation in response to mating pheromone. [36] Module 13 contains RING11, PSF2, ABCB3, ATP binding cassette sub family B member 3, NGS 17 and TAP1, PSF1, ATP binding cassette sub family B member 2. The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. [49] ABC proteins transport various molecules across extra- and intra-cellular membranes. This protein is a member of the MDR/TAP subfamily. Members of the MDR/TAP subfamily are involved in multidrug resistance. The protein encoded by this gene is involved in the pumping of degraded cytosolic peptides across the endoplasmic reticulum into the membrane-bound compartment where class I molecules assemble. Mutations in this gene may be associated with ankylosing spondylitis, insulin-dependent diabetes mellitus, and celiac disease. [49]

4. CONCLUSION

With the development of high-throughput techniques, protein interaction network analysis attracts more interest because the systematic approach makes it a significant tool for the detailed analysis of certain functions and processes based on specific sub-networks. Although, the computational predictions for PPIs are complimentary to the *in vivo* experiment, this study provides an efficient way to elucidate possible mechanism of bacterial Universal stress protein and the protein-protein interactions information is connected to the protein secretion pathway, apoptosis, cell division, cell shape and gene regulation. These results will serve as a unique resource to provide a physical map of protein profile of bacteria under stress condition, for further analysis of signal transduction, drug targeting and the development of novel therapeutics.

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